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KRITISCHE STELLUNGNAHME ZU DER ARBEIT VON POGLAYEN-NEUWALL „UNTERSUCHUNGEN DER KIEFERMUSKULATUR UND DEREN INNERVATION BEI SCHILDKRÖTEN"

VON

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Aus dem Anatomischen Institut der Universität Greifswald (Direktor Prof. Dr. Dr. R. N. Wegner).

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I. VORWORT

Zur gleichen Zeit als Ivo POGLAYEN-NEUWALL seine „Untersuchungen der Kiefermuskulatur und deren Innervation bei Schildkröten" in der Acta Zoologica, Arg. 34, Häft 3, 1953 veröffentlichte, befand sich meine I. Mitteilung „Beiträge zur Kiefermuskulatur der Schildkröten" im Druck. Ich hatte unabhängig von P.N. meine Untersuchungen vom Juli 1951 bis Januar 1954 im Anatomischen Institut der Universität Greifswald ausgeführt. Während POGLAYEN-NEUWALL außer der Kiefermuskulatur auch über die Innervation derselben berichtet und ein exaktes Schema des Nervus trigeminus gibt, ging meine Zielsetzung dahin, eine genaue morphologische Analyse der Kiefermuskulatur unter Herausstellung des M.pterygoideus zu geben, verbunden mit einer

zusammenhängenden Fasciendarstellung. Da ich beim Studium seiner Arbeit feststellen konnte, daß bei ähnlicher Themastellung unsere Ergebnisse zum Teil etwas unterschiedlich sind, entschloß ich mich, in Form einer Arbeit zu seinen interessanten Befunden Stellung zu nehmen. Durch die Gründlichkeit und den Umfang seiner Arbeit bewogen, hielt ich es für angebracht, meine Untersuchungsergebnisse P.N.'s Befunden gegenüberzustellen. So mag diese Arbeit dazu beitragen, den Arbeitskomplex über die Kiefermuskulatur der Schildkröten abzurunden und Lücken, die sich aus einer verschiedenen Thematik, Untersuchungsmethoden, dem unterschiedlichen Material etc. ergeben, füllen zu helfen. Begünstigend für die Gegenüberstellung ist, daß P.N. das von RUGE und LUTHER aufgestellte und von LAKJER-LUBOSCH übernommene Einteilungsprinzip benutzt hat, welches ich auch meinen Ausführungen zugrundegelegt habe, mit dem ich mich jedoch nicht immer einverstanden erklären konnte. Nach Präparationen des ganzen mir zur Verfügung stehenden Schildkrötenmaterials und der Zusammenstellung der Befunde in einer 2. und 3. Mitteilung, habe ich die hier vorliegende Arbeit begonnen.

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II. EINLEITUNG

Gegenüberstellung des von POGLAYEN-NEUWALL und von SCHUMACHER zur Untersuchung benutzten Materials

Da die Entscheidung über grundsätzliche Unterschiede sehr häufig von der Auswahl und dem Umfange des jeweils bearbeiteten Materials abhängt, so gebe ich einleitend eine Gegenüberstellung des von P.N. und mir durchpräparierten und benutzten Materials.

Es ist wohl jedem Autor von vornherein klar, daß für eine solche Aufgabe, wie sie sich beide Verfasser gestellt haben, das Material gar nicht umfangreich genug sein kann. Aber trotzdem werden der Materialbeschaffung auch in großen Museen nur zu enge Grenzen gesetzt sein. Ich glaube bemerken zu können, daß mir mein Material bis auf wenige Ausnahmen einen genügenden Umfang bietet, um in die Verhältnisse der Kiefermuskulatur der Schildkröten einen Einblick geben zu können.

Es ist mir ein Bedürfnis, an dieser Stelle meinem hochverehrten Lehrer, Herrn Prof.Dr. med. et phil. R.N.WEGNER, meinen ergebensten Dank dafür auszusprechen, daß er mir in der großzügigsten Weise seine ganze private Sammlung sowie seine außerordentlich umfangreiche, kostbare Bibliothek zur Verfügung stellte, und daß er meine Untersuchungen mit dem regsten Interesse verfolgte und mir immer mit seinem wertvollen Rat und seiner steten Hilfsbereitschaft zur Seite stand.

In besonderer Weise danke ich dem Kustos und Leiter der Reptilienabteilung des Zoologischen Museums der Humboldt-Universität Berlin, Herrn Dr.Heinz WERMUTH, der mich

nicht nur mit sehr schönem Material unterstützt hat, sondern die genaue Bestimmung der meisten hier untersuchten Schildkröten vorgenommen und mir die neueste jetzt gültige zoologische Nomenklatur mitgeteilt hat. Ich verdanke ihm auch sonst manch wertvollen Rat.

Mein besonderer Dank gilt auch dem Direktor des Zoologischen Staatsinstitutes und Zoologischen Museums, Hamburg, Herrn Prof. Dr. B. KLATT, dem Direktor des Zoologischen Gartens, Leipzig, Herrn Prof. Dr. SCHNEIDER † und Herrn Dr. DATHE, dem Direktor des Zoologischen Gartens Berlin Treptow, dem Direktor des Naturhistorischen Museums Stralsund, Herrn Prof. Dr. DIBBELT † für die großzügige Überlassung von wertvollem Schildkrötenmaterial.

In besonderem Maße möchte ich auch dem akademischen Zeichner des Anatomischen Instituts der Universität Greifswald, Herrn Johannes SCHUBERT, für die Zeichnungen danken, die er mit viel Geduld und Liebe angefertigt hat.

Das von POGLAYEN-NEUWALL 1953 angegebene Material[1]:

Fam. *Chelydridae*	*Chelydra serpentina* 2 Expl.
Fam. *Kinosternidae*	*Sternotherus odoratus (Cinosternum odoratum)*
„	*Kinosternon scorpioides*
„	*Kinosternon leucostomum*
Fam. *Emydidae*	*Chrysemys picta*
„	*Pseudemys scripta troostii* 2 Expl.
„	*Malaclemys terrapin littoralis*
„	*Graptemys pseudogeographica (Malaclemys lesueurii)*
„	*Terrapene carolina triunguis (T.cinosternoides)*
„	*Cuora amboinensis (Cyclemys a.)* 3 Expl.
„	*Clemmys caspica*
„	*Clemmys guttata*
„	*Emys orbicularis (E.lutaria, E.europeae)* 2 Expl.
Fam. *Testudinidae*	*Testudo graeca (T.ibera)* 2 Expl.
Fam. *Cheloniidae*	*Caretta caretta (Thalassochelys c.)* 2 Expl.
Fam. *Dermochelyidae* ..	*Dermochelys coriacea (Sphargis c.)*
Fam. *Trionychidae*	*Amyda sinensis (Trionyx s.)*
Fam. *Pelomedusidae* ..	*Pelusios niger (Sternothaerus niger)*
Fam. *Chelidae*	*Chelodina longicollis* 3 Expl.
„	*Batrachemys nasuta (Rhinemys n.)*

Das von SCHUMACHER 1954 benutzte Material

Fam. *Chelydridae*	*Macrochelys temminckii* (TROOST) — Spirit.-Hals-Kopf-Präparat u.Schädel, Col. WEGNER

[1] Die Materialübersicht ist etwas umgegliedert worden, um eine bessere und zweckmäßigere Gegenüberstellung zu meiner Materialzusammenstellung zu ermöglichen. Die unter Fam.*Testudinidae* aufgeführten Schildkröten gehören der Fam.*Emydidae* mit Ausnahme von *Testudo graeca* an, ich habe daher statt Fam.*Testudinidae* Fam.*Emydidae* gesetzt. Bei *Malaclemys pileata littoralis* habe ich den neuen Namen *Malaclemys terrapin littoralis* und bei *Terrapene triunguis* die neue Bezeichnung *Terrapene carolina triunguis* benutzt.

Fam. *Platysternidae* .. *Platysternon megacephalum* GRAY — Spirit.-Präp. u.Schädel,Col.WEGNER

Fam. *Emydidae* ..... *Clemmys caspica rivulata* (VALENCIENNES) — Spirit.-Präp.,Col.WEGNER

„ ..... *Emys orbicularis* (LINNÉ) — 5 Spirit.-Präp.u.2 Schädel,Anat.Inst.Greifswald u.Col.WEGNER

„ ..... *Graptemys geographica* (LE SUEUR) — 2 Spirit.-Präp.u.Schädel,Col.WEGNER

„ ..... *Hardella thurjii* (GRAY) — Spirit.-Präp.,Col.WEGNER

Fam. *Testudinidae* .... *Testudo elephantopus* HARLAN — 2 Spirit.Präp. Kopf- u. Hals-Kopfpräparate u. 3 Schädel,Col. WEGNER

„ .... *Testudo gigantea* SCHWEIGGER — 3 Schädel,Col. WEGNER

„ .... *Testudo graeca* LINNÉ — 4 Spirit.-Präp. u. 2 Schädel.Anat.Inst.Greifswald,Col.WEGNER

Fam. *Cheloniidae* ..... Caretta caretta (LINNÉ — Spirit.-Kopf-Präp. u. 2 Schädel,Col.WEGNER

„ ..... *Chelonia mydas* (LINNÉ) — 2 Spirit.-Kopf-Präp. u. 6 Schädel,Zool.Museum Berlin, Zool. Staatsmuseum Hamburg, Col.WEGNER

„ ..... *Eretmochelys imbricata* (LINNÉ) — 2 Schädel,Col. WEGNER

Fam. *Trionychidae* .... *Amyda ferox* (SCHNEIDER) — Schädel,Col.WEGNER

„ .... *Amyda sinensis* (WIEGMANN) — 4 Spirit.-Präp., Anat.Inst.Greifswald

„ .... *Dogania subplana* (GEOFFROY DE St. HILAIRE) — Spirit.-Präp.,Col.WEGNER

„ .... *Trionyx punctatus* (BONNATERRE) — Spirit.-Präp., Col.WEGNER

Fam. *Pelomedusidae* .. *Pelomedusa subrufa* (LACÉPÈDE) — Spirit.-Kopf-Präp-,Zool.Museum Berlin

„ .. *Pelusios sinuatus* (SMITH) — Spirit.-Kopf.-Präp.u. Schädel,Zool.Museum Berlin

„ .. *Pelusios subniger* (LACÉPÈDE) — 2 Spirit.-Kopf.-Präp.u.2 Schädel,Zool.Museum Berlin,Zool.Staatsmuseum Hamburg,Col.WEGNER

„ .. *Podocnemis expansa* (SCHWEIGGER) — 2 Spirit.-Kopf.-Präp.u.8 Schädel,Zool. Staatsmuseum Hamburg,Zool.Museum Berlin,Col.WEGNER

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Fam. *Pelomedusidae* .. *Podocnemis madagascariensis* (GRANDIDIER) — Schädel,Col.WEGNER

" .. *Podocnemis unifilis* (TROSCHEL) — Spirit.-Hals.-Kopf.-Präp.,Zool.Museum Berlin

Fam. *Chelidae* ..... *Chelodina longicollis* (SHAW) — Präp.u.Schädel, Zool.Museum Berlin

„ „ ..... *Hydromedusa tectifera* COPE — Präp.u.Schädel, Zool.Museum Berlin

„ „ ..... *Emydura krefftii* (GRAY) — Spirit.-Präp.Col.WEGNER

III. DER M.ADDUCTOR MANDIBULAE

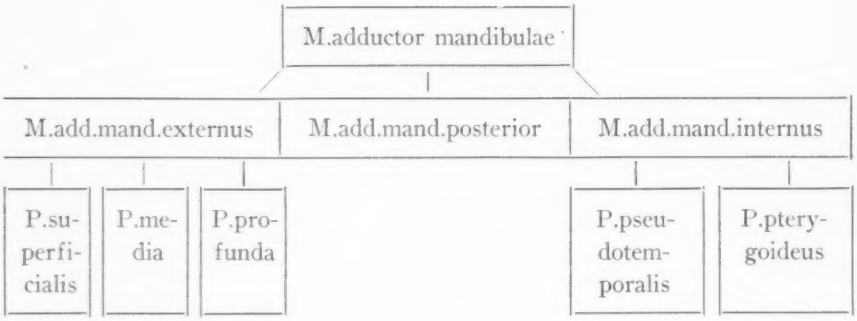
Obgleich ich meinen Mitteilungen die gleiche Nomenklatur zugrundegelegt habe wie P.N., bin ich in der Abgrenzung der Portionen doch abweichender Meinung, woraus ein Teil unserer unterschiedlichen Ergebnisse abzuleiten ist.

Der M.adductor mandibulae läßt zwei Hauptmuskeln erkennen, die vollkommen voneinander zu trennen sind, den M.adductor mandibularis externus und den M.adductor mandibularis internus. Der M.add.mand.ext., welcher der weitaus kräftigere ist, füllt die ganze obere und einen Teil der unteren Temporalgrube aus. Er läßt sich in drei Portionen unterteilen, eine Portio superficialis, medialis und profunda. Bei den Trionychiden tritt noch ein zusätzlicher Muskel in Erscheinung, der außerhalb der Temporalgrube vom knöchernen Jochbogen zur Mandibel verläuft. Er ist von OGUSHI 1926 als M.masseter und von mir 1955 als M.zygomatico-mandibularis bezeichnet worden.

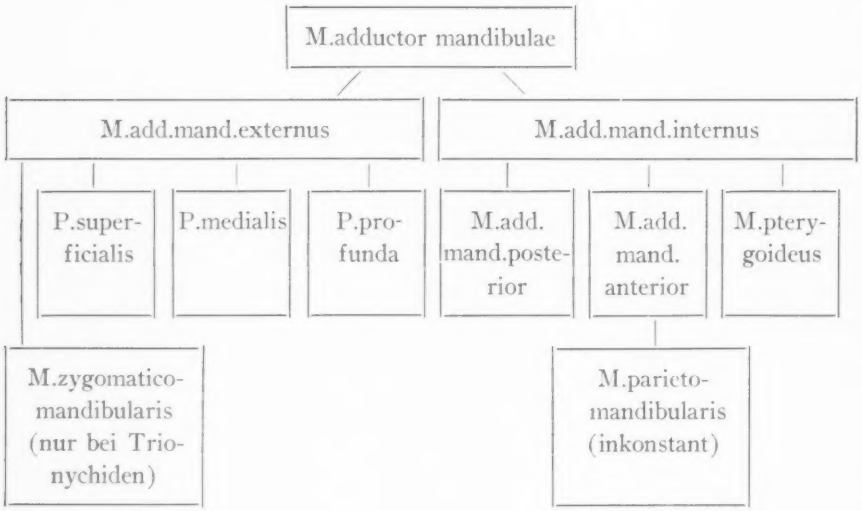
Den M.add.mand.int. fasse ich als Muskelkomplex auf und stelle ihn dem M.add.mand.ext. gegenüber. Ich habe nicht an der von LAKJER und P.N. gebrauchten Aufteilung festgehalten (M.add.mand.posterior und internus), da eine vollständige Gliederung in einzelne Muskelindividuen nicht gelingt. Der M.add.mand.int. breitet sich nur in der unteren Temporalgrube aus, seine Zugrichtung unterkreuzt die des M.add.mand.ext.. Den Internuskomplex unterteile ich in einen M.add.mand. posterior, M.add.mand.anterior und einen M. pterygoideus. Die Bezeichnungen „posterior" und „anterior" sind auf Grund der Lagebeziehungen zum Foramen nervi trigemini gewählt. Das von P.N. als M.add.mand.post. aufgeführte Muskelstück ist mit dem von mir genannten identisch, nur daß ich es als Teil des M.add.mand.int. ansehe. Als M.add.mand. ant. habe ich den Muskelabschnitt bezeichnet, der vor dem For.nervi trigemini liegt und der von LAKJER und P.N. als P.pseudotemporalis des M.add.mand.int. beschrieben wurde. Die Bezeichnung „M.pseudotemporalis" habe ich zugunsten der Benennung „M.parieto-mandibularis" ganz fallen lassen. Dieser Muskel ist

inkonstant, besitzt zwei Köpfe, dessen einer meinem M.add.mand.ant. aufliegt oder mit ihm verwachsen ist, dessen zweiter im Unterkiefer eingeschlossen liegt und von P.N. als „M.intramandibularis" bezeichnet wurde. Über die Ausbreitung des M.pterygoideus besteht weitgehende Übereinstimmung. Wo nötig, habe ich diesen noch in Portionen unterteilt. Die Bezeichnung „Musculus" für die untergegliederten Muskelstücke des Internuskomplexes soll nicht irreführen. Ich habe sie nur benutzt, um die genannten Muskelteile noch weiter zu unterteilen und sie mit der Bezeichnung „Portio" belegen zu können. Zur besseren Übersicht gebe ich eine schematische Gegenüberstellung der von P.N. und mir gebrauchten Nomenklatur.

POGLAYEN-NEUWALL:



SCHUMACHER:



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KRITISCHE STELLUNGNAHME ZU DER ARBEIT

a) DER M.ADDUCTOR MANDIBULARIS EXTERNUS

Der M.add.mand.ext. ist, wie bereits hervorgehoben, der weitaus kräftigste Kaumuskel der Schildkröten. Sein Ursprungsgebiet liegt in der ganzen oberen, z.T. auch in der unteren Temporalgrube. In der Tiefe des Muskels findet sich eine ansehnliche Binnensehne[1], die reichlich Differenzierungen aufweisen kann und dem größten Teil der Muskelfasern ein-, zwei- oder allseitig zum Ansatz dient. Dadurch erhält der M.add.mand.ext. eine gefiederte Struktur. Wir wissen, daß das Prinzip der Fiederung eine physiologische Querschnittsvergrößerung zur Folge hat, die gleichbedeutend mit einer stärkeren Kraftentfaltung bei minimalster Raumbeanspruchung ist. Diese Fiederung gibt nun wiederum Anlaß, den Muskel in Portionen zu unterteilen.

Ich schließe mich ganz der Meinung von P.N. an, daß sich der M.add. mand.ext. nicht in selbständige isolierbare Muskeln unterteilen läßt. Gleich ihm habe auch ich die Bezeichnung Portionen benutzt. P.N. schreibt zu den allgemeinen Lageverhältnissen der drei Portionen folgendes: „Die P.supf. füllt in einer Sagittalebene den Großteil der unteren Schläfengrube aus. Sie entspringt medial des Os jugale und Os quadratojugale. Die P.media schließt medio-caudal an die P.supf. an und entspringt oft mit den dorso-caudalen Fasern an der äußeren knöchernen Umrahmung der oberen Schläfengrube, wodurch sie auch dorsal der ersterwähnten Portion zu liegen kommt. Die P. profunda schließlich liegt medial von beiden Portionen und entspringt an der inneren knöchernen Umrahmung der oberen Temporalgrube, so daß auch diese Portion die P.supf. dorsal überragt."

Bevor ich im einzelnen auf die Befunde von P.N. eingehe, möchte ich überblicksmäßig meine eigene Beobachtung über Lage und Form der drei Muskelportionen hier wiedergeben.

Die Muskelfasern, die ich als P. superficialis bezeichne, befinden sich am weitesten lateral, und wenn man das knöcherne Schläfendach eines stegalen Schildkrötenvertreters entfernt, auch am oberflächlichsten, immer lateral von der Binnensehne des M.add.mand.ext.. Die Fasermasse breitet sich in der oberen und unteren Temporalgrube aus. Sie entspringen von der lateralen Hälfte der oberen Temporalgrube und des Schläfendaches. Der Ansatz erfolgt zum größten Teil an der Sehne des M.add.mand.ext. und zwar im Prinzip an deren Lateralfläche und an der Lateralseite des Proc.coronoides mandibulae. Häufig besitzt die P.supf. noch eine rostrale Lamelle *(Testudo elephantopus, Pelusios* usw.), diese liegt unterhalb des Jochbogens und bildet eine oberflächliche rostrale Lage des M.add.mand.ext.. Die P.supf. besitzt im allgemeinen eine geringere Ausdehnung als die massivere P.prof..

[1] Die Binnensehne bezeichne ich als Sehne des M.add.mand.ext. oder kurz Adductoren- oder Externussehne.

Die P. medialis ist immer die kleinste Portion des Externus und nimmt nur einen kleinen ventralen Bezirk in der unteren Temporalgrube ein. Ihr Aussehen ist fächerförmig. Die ventralsten Fasern verlaufen horizontal parallel zum Unterkiefer, richten sich langsam auf und nehmen allmählich eine immer steilere Stellung ein, um schließlich an die nahezu verticale Faserverlaufsrichtung der P.supf. Anschluß zu gewinnen und ohne scharfe Grenze in dieselbe überzugehen. Das Ursprungsgebiet dieser Portion ist am wenigsten Variationen unterworfen und beschränkt sich im allgemeinen auf die Rostralfläche des Quadratum. Die Insertion erfolgt auf der Lateralfläche des Proc. coronoides und der Adductorensehne.

In Anbetracht des völlig fließenden Überganges zur P.supf. erhebt sich überhaupt die Frage, ob man letztere als selbständige Portion ansehen darf. Zu dem medial gelegenen M.add.mand.int. ist sie durch ihre eigene, bzw. durch die Fascie des benachbarten Muskels immer scharf getrennt.

Die P. profunda ist im allgemeinen am stärksten ausgebildet und unterliegt sowohl in ihrer äußeren Form als auch in ihrer inneren Architektur Variationen. Medial von der Adductorensehne gelegen, nimmt sie in der Länge und Breite durchschnittlich den größten Raum in der hinteren Temporalgrube ein. Sie ist häufig um das drei- bis vierfache breiter als die P.supf.. Sie entspringt von der medialen Hälfte der oberen, z.T. auch der unteren Temporalgrube und der medialen temporalen Bedachung. Der größte Teil der Fasern inseriert im wesentlichen an der Medialseite der Adductorensehne. Die Größe und Ausdehnung der P.prof. ist im allgemeinen von der Ausbildung der Crista supraoccipitalis abhängig, und da diese im allgemeinen stärker nach caudal ausgezogen ist als das Supratemporale, ist die P.prof. stärker als die P.supf. Charakteristisch für die P.prof. ist der caudale Muskelwulst, der die Temporalgrube meist nach caudal überragt.

Der M. zygomatico-mandibularis ist vollkommen selbständig und kommt nur bei den Trionychiden vor. Funktionell bildet er mit dem M.add. mand.ext. eine Einheit, morphologisch läßt er sich jedoch keiner Externusportion zuordnen. Er nimmt seinen Ursprung vom Jochbogen und inseriert an der Lateralfläche der Mandibel.

1. Zu den Befunden von P. N. über den M. adductor mandibularis externus

Poglayen-Neuwall schreibt, daß der M.add.mand.ext. von einer starken bindegewebigen Fascie bedeckt werde (Temporalfascie nach Lakjer), die dem Muskel eng anliege.

Ich fand, daß die Temporalfascie dem Muskel nicht eng anliegt, sondern durch lockeres Bindegewebe von diesem getrennt ist. Dieses Bauprinzip läßt sich aus der Funktion der Temporalfascie und dem Adductorenmuskel

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erklären. Die Fascia temporalis überspannt den dorsalen Ausschnitt der oberen Temporalgrube als feste, derbe, bindegewebige Membran und bietet den Schildkröten mit reduziertem Schläfendach einen Ersatz für den verlorengegangenen Knochen. Um die Funktion eines Knochens zu übernehmen, muß sie verschiedenen mechanischen Beanspruchungen standhalten können und dem M.add.mand.ext. Ursprungsmöglichkeiten für seine Muskelfasern geben. Durch die Kontraktion des M.splenius capitis, der an ihrem freien Caudalrand inseriert, wird sie in einen erhöhten Spannungszustand versetzt, so daß sie vollends den Charakter einer straffen Membran erhält. Auf ihrer Dorsalseite ist sie mit der äußeren Haut fest verwachsen. Die Temporalfascie hat eine ausgesprochene Halte- und keine Hüllfunktion, so ist es auch verständlich, daß sie dem ständig in Bewegung befindlichen M.add.mand.ext. nicht eng anliegen darf. Die Fascie des M.add.mand.ext. hingegen umkleidet den Muskel und begleitet ihn von seinem Ursprung bis zu seinem Ansatz.

Von der Temporalfascie schreibt P.N., daß sie sich größtenteils an der knöchernen Umrahmung der Schläfengrube befestige, medial am Rostralabschnitt des Squamosum und Quadratum, am Caudalrand des Postfrontale und an der Dorsalkante des Parietale. Das Supraoccipitale wird von ihm nicht erwähnt. Mit wenigen Ausnahmen habe ich jedoch auch eine Befestigung am Supraoccipitale finden können. Die von P.N. am Quadratum erwähnte Insertion konnte ich nur in solchen Fällen beobachten, wo kein Jochbogen vorhanden ist *(Chelodina longicollis* und *Hydromedusa tectifera)*. Hier bildet das Supratemporale und Quadratum in der oberen Schläfengrube eine laterale plateauartige Erhebung. Eine Knochenkante findet sich nicht, so daß die obere Temporalgrube nach lateral offen ist. Wo ein knöcherner Jochbogen existiert, bilden das Supratemporale und Squamosum eine laterale Kante, die das Quadratum bedeckt und der oberen Temporalgrube den Charakter einer Grube verleiht. An dieser Kante inseriert die Temporalfascie. Ein Einstrahlen in die bindegewebige Mundwinkelhaut, wie es P.N. erwähnt und wie es aus seiner Abb.1,a, *(Batrachemys nasuta)* ersichtlich ist, habe ich nicht finden können. Bei Schildkröten mit fehlendem Temporalbogen ist ein mehr oder weniger breiter Kontakt zur äußeren Mundplatte dadurch vorhanden, daß diese medial an der Tp.-Fascie emporsteigt. Die Fascie inseriert an einem derben Band, welches vom Quadratum entspringt und am Jugale und Maxillare ansetzt. (Lig. quadrato-jugo-maxillare). Bei Schildkröten vom zygalen Typ erreicht die Tp.-Fascie die Mp. trotz steilen Anstieges gar nicht; sie inseriert, wie eben angegeben. Eine Zweiteilung der Fascie habe auch ich in keinem Falle beobachtet. Eine Temporalfascie als lateraler Abschluß der unteren Temporalgrube gibt es bei Schildkröten vom zygalen und stegalen Typ nicht. Als Abdeckung des lateralen Temporalfensters fand ich außer der Fascie des M.add.mand.ext. ein filziges Bindegewebe, das mit dem Unterhautbindegewebe identisch ist,

und das ich infolge seiner Ausbreitung und seiner derben Konsistenz als Membrana quadrato-squamoso-jugo-maxillaris bezeichnet habe.

Die Sehne des M.adductor mandibularis externus findet bei P.N. als Binnensehne nur eine kurze Erwähnung. Er schreibt, daß die Sehne im vorderen Teil der oberen Schläfengrube, dem Prooticum, an dessen Kante sie bei den Kaubewegungen gleitet, anliege und nach ventral in die untere Schläfengrube biege, dabei ihre Flächen oft bis zu 90° in sagittaler Richtung drehend und dorsal am Coronoid (Complementare nach Fuchs, 1931) und zusätzlich auch am Dentale *(Chelydra)*, am Dentale und Supraangulare *(Caretta)* oder auch an der Gaumenhaut medial vom Coronoid *(Chelodina, Batrachemys)* inserieren soll.

Ich fand, wie schon bekannt, daß die Sehne nicht nur dem Prooticum allein, sondern auch dem Quadratum aufliegt, sofern ihr nicht ein flügelartiger Fortsatz des Pterygoidbeines als Unterlage dient. (Diese knöcherne Unterlage habe ich als Proc.trochlearis bezeichnet).

Die hintere Wand der unteren bildet mit dem Boden der oberen Temporalgrube häufig einen Winkel, der kleiner ist als 90°. Besonders bei dem dachartig nach rostral vorspringenden Quadratum und Prooticum der Trionychiden ist er sehr spitz ausgebildet. So muss also die darüber hinwegziehende Sehne eine entsprechende dorso-ventrale Richtungsänderung, bezw. Abknickung erfahren, so dass wir also ausser der Drehung um die eigene Achse oder in sagittaler Richtung, wie P.N. schreibt, noch einen Knickungswinkel berücksichtigen müssen. Bei einigen Schildkröten habe ich ihn gemessen, so beträgt er bei *Chelone* und *Caretta* etwa 130°. Bei *Podocnemis* und *Pelusios*, das sind Vertreter mit pterygoidalem Anteil am Proc.trochlearis, ist er größer und beträgt 140—150°, obgleich der Übergang von der unteren in die obere Temporalgrube genau so scharfrandig ist wie bei den erstgenannten. Bei den Trionychiden fand ich die stärkste Abknickung, hier betrug der Winkel etwa 100°. Auch die Insertion der Adductorensehne am Unterkiefer hat Einfluß auf die Größe des Sehnenknickungswinkels. Mit einer Rostralverlagerung des Proc.coronoides muß der Winkel stumpfer, bei einer Caudalverlagerung spitzer werden, unabhängig von dem Winkel, den die hintere Wand der unteren mit dem Boden der oberen Temporalgrube bildet.

Nach meinen Feststellungen erfolgt der Ansatz der Sehne bei *Caretta* nicht nur am Dentale und am Supraangulare, gleichfalls konnte ich eine Insertion an der Gaumenhaut nicht finden. Die Mundhaut steht zwar in enger Beziehung zur Adductorensehne, jedoch inseriert nicht die Adductorensehne an der Gaumenhaut, sondern die innere Mundplatte heftet sich zum Teil recht fest an die Rostral- und Medialseite der Adductorensehne.

Die Befestigung der Sehne des M.add.mand.ext. erfolgt recht breitflächig am Proc.coronoides mandibulae, die Variationen im Ansatz sind bei den einzelnen Schildkrötenarten unbedeutend. Sie inseriert lateral am Dentale, z.T. noch am Supraangulare, dorsal und medial am Coronoid und Goniale.

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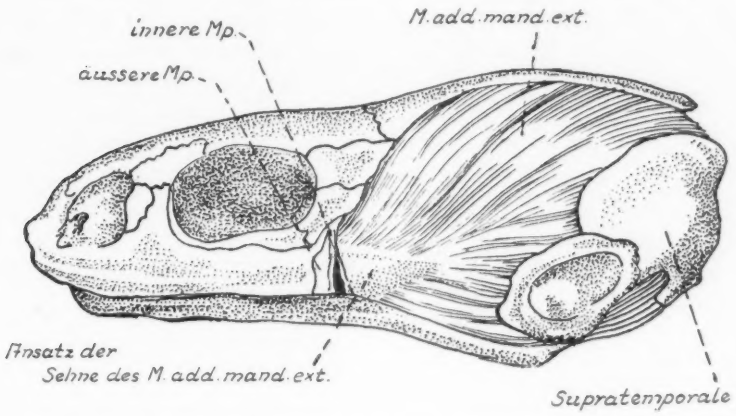

Abb. 1. Laterale Ansicht eines Kopfes von *Chelodina longicollis.* (SHAW) Wie man sieht, besitzt der M.adductor mandibularis externus keine natürliche Abgrenzung in Portionen.

Die Unterteilung des Add.ext. in drei Portionen sei fast immer deutlich, schreibt P.N., es kämen nur selten Übergänge vor. Ich fand, daß sich die P.superficialis in keinem Falle von der P.medialis scharf trennen läßt. Die Abgrenzung beider voneinander kann nur eine willkürliche sein. Manchmal ist auch die Abgrenzung der P.profunda von der P.superficialis im Rostralbereich nicht ganz eindeutig, obgleich die P.superficialis und die P.profunda bei der äußeren Betrachtung des Muskels im allgemeinen gut zu unterscheiden sind. Die Trennung wird in den meisten Fällen schon äußerlich durch den Verlauf der Adductorensehne markiert, welche mit Ausnahme von *Chelodina longicollis, Hydromedusa tectifera* und *Emydura krefftii* einen mehr oder weniger tiefen Sulcus im M.add.mand.ext. erkennen läßt, der mit lockerem Bindegewebe ausgefüllt ist und der Einlagerung und Polsterung der temporalen Gefäße dient. Auch P.N. vermißte bei *Chelodina* eine deutliche Trennung der P.superficialis von der P.medialis und P.profunda und bei *Batrachemys,* die *Hydromedusa* etwa gleichzusetzen ist, einen Übergang zwischen der P.superficialis und profunda in deren rostralen Abschnitten.

Bei *Chelodina* und *Emydura* sind die Verhältnisse derart, daß erst nach Abheben des M.splenius capitis im caudalen Bereiche zwei Muskelbäuche deutlich werden, medial der caudale Wulst der P.profunda, lateral der etwas stärkere der P.superficialis. Bei *Chelodina* steigt der M.add.mand.ext. fließend aus der unteren in die obere Temporalgrube auf, legt sich mit seiner lateralen Muskelhälfte der P.superficialis um den caudalen Fortsatz des Supratemporale hakenförmig herum und greift bis an die Unterfläche desselben. Damit nimmt der Muskel, jedenfalls auf die P.superficialis bezogen, eine S-Form an, dessen stärkerer Bogen unter dem Supratemporale seinen Anfang nimmt, während der flachere Bogen in der unteren Schläfengrube zu finden ist. Im Gebiet

der unteren Temporalgrube, etwas oberhalb vom Proc.coronoides, wird bei *Chelodina*, weniger bei *Hydromedusa*, ein weißlich-gelblich glänzendes Knötchen sichtbar, welches durch die Lage der Cartilago transiliens in der Adductorensehne bedingt ist. Unmittelbar vor dieser Auftreibung liegt die innere Mundplatte.

Will man eine Einteilung vornehmen, so muß man den äußerlich ungegliederten Muskel entsprechend der Lage und des Verlaufes seiner Fasern in die uns bekannten Muskelportionen zerlegen. Die Fasern des ventralsten Abschnittes verlaufen in horizontaler Richtung parallel zum Unterkiefer. Sie entspringen von der Rostralfläche des Quadratum, ziehen konvergierend nach rostral und inserieren am Supraangulare sowie am caudalen Rande des Coronoids. Nach dorsal richten sie sich allmählich auf, ohne jedoch eine vollkommene Verticalstellung zu erreichen. Sie geben das Bild eines Fächers und würden der oben beschriebenen P.medialis entsprechen. Die rostralsten nahezu vertical verlaufenden Fasern wären der P.superficialis und P.profunda zuzuordnen, an denen sich im unteren Abschnitt die innere Mundplatte anlegt.

Verfolgt man nach Resektion des Schläfendaches bei *Emydura krefftii* den Muskel von rostral nach caudal, so nimmt man in seinem caudalen Abschnitt eine Gabelung wahr, deren medialer Schenkel der P.profunda und deren lateraler Schenkel der P.superficialis entspricht. Die Faseranteile der letzteren haben ihr Ursprungsareal rostral am Temporaldach und zwar an der Innenseite des caudalen Randes des Postfrontale und vom lateralen Rande des Parietale. Die Crista supraoccipitalis dient dem verhältnismäßig kleinen Muskelwulst der P. profunda zum Ursprung, während der stärkere Muskelballen der P.superficialis im caudalen Abschnitt vom Supratemporale entspringt. Die der P. profunda entsprechenden Fasern laufen fast horizontal in der üblichen Richtung von caudal medial nach rostral lateral, die der P.superficialis zuzuordnenden konvergieren mit letzteren wie üblich, indem sie von caudal lateral nach rostral medial ziehen. Bei *Hydromedusa tectifera* kommt es im caudalen Abschnitt nicht zur Ausbildung von getrennten Muskelbäuchen. Offensichtlich wird es durch die caudale Knochenspange verhindert. Die verhältnismäßig langen Muskelfasern ziehen von caudal medial nach lateral ventral, die rostralsten sind unmittelbar hinter der inneren Mundplatte gelegen und mit dieser mittels Bindegewebe eng verbunden. Der mittlere Faseranteil zieht in Richtung der Temporalgrube von caudal medial dorsal nach rostral lateral und ventral. Alle diese Fasern entsprechen in ihrer Lage und Verlaufsrichtung der P.profunda. Die lateralen Faserzüge verlaufen von caudal nach rostral, so daß der ganze Muskel ein fächerförmiges Aussehen erhält. Der M.add.mand.ext. entspringt von der Temporalfläche des Parietale und Supraoccipitale, die caudalsten Fasern von der caudalen Knochenspange. Letzterer begrenzt ihn nach caudal, jedoch nur oberflächlich. Das Occipitale bildet mit seiner ganzen Occipitalfläche ein weiteres Ursprungsfeld. Die Temporalfläche des Opisthoticum und Supratempo-

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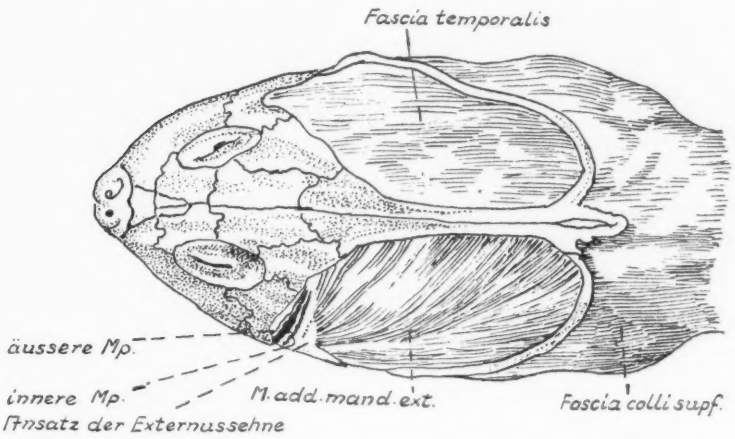

Abb. 2. Dorsale Ansicht eines Kopfes von *Hydromedusa tectifera* COPE nach Entfernen der äusseren Haut. Rechts ist die Fascia temporalis belassen, links sieht man den M. adductor mandibularis externus. Eine Untergliederung in Portionen ist nicht zu erkennen.

rale dienen den Muskelfasern als Ursprung. Ein weiteres Areal bildet die Unterfläche des caudalen Knochenspornes vom Supratemporale. Alle Fasern konvergieren nach rostral und medial, um an der in der Tiefe gelegenen Sehne oder direkt an der Mandibel zu inserieren. Auch bei der Tiefenpräparation zeigt sich das Fehlen einer Unterteilung in einzelne Portionen.

2. Zur P. superficialis

P.N. schreibt, daß sich die P.superficialis in der Regel auf das laterale Gebiet der unteren Temporalgrube beschränke, nur bei *Kinosternon* und *Cuora* reiche der Ursprung bis weit in die obere Schläfengrube. Sie fülle die untere Schläfengrube nahezu in ihrer ganzen Breite aus, und nur in deren caudalsten Teil lasse sie Fasern der P.media durchblicken mit Ausnahme von *Chelydra, Kinosternon, Sternotherus* und *Amyda,* bei denen die P.superficialis so ausgebildet sei, daß keine Mediusfasern vor Abtragung der Superficialisschicht im ventro-lateralen Abschnitt der unteren Schläfengrube sichtbar seien. Nach P.N. entspringen zahlreiche Fasern der P.superficialis von der Medio-Rostralfläche des Quadratum, die Masse der Fasern jedoch an der Temporalfascie medial des Quadrato-Jugale (Squamosum nach LAKJER) und Jugale, bei Fehlen dieses Bogens an der Temporalfascie entlang eines Streifens auf ungefährer Höhe des Dorsalrandes von Tympanon und Orbita. Oft käme als zusätzliches Ursprungsgebiet für die rostralen Fasern die Caudo-Medialfläche des Postfrontale hinzu, wie bei *Clemmys, Emys, Malaclemys, Pseudemys* und *Chelydra,* manchmal träte auch der Dorso-Medialrand des Squamosum hinzu, so bei *Cuora* und *Kinosternon.*

Da unsere Meinungen über die grundsätzliche Einteilung der Portionen des M.add.mand.ext. auseinandergehen, wird man natürlich auch über Ursprung und Ansatz keine Einigung erzielen. Die von P.N. angegebene Medio-Rostralfläche des Quadratum ist meines Erachtens als Ursprungsgebiet völlig auszuschließen, da dieses das konstante Ursprungsareal des M.add.mand.post. ist, der dem M.add.mand.int. angehört. Es wird dieser Knochenbezirk von Anteilen des M.add.mand.ext. nicht erreicht und die P.superficialis käme für einen so tiefen Ursprung schon gar nicht in Frage. Daß die Hauptmasse der Fasern von der Temporalfascie entspringen soll, habe ich nicht gefunden. Die Gesamtzahl der von der Temporalfascie entspringenden Muskelfasern ist verhältnismäßig gering. Um nicht auf alle Einzelheiten einzugehen, habe ich die Vielfalt der Ursprünge der P.superficialis in knapper übersichtlicher Form zusammengestellt.

Ursprung der P.superficialis

Parietale:	
lateralster Parietalfortsatz:	*Pelusios, Platysternon, Emydura, Chelone, Caretta*
Postfrontale:	
Innenfläche: (vorwiegend caud. Bezirk)	*Platysternon, Pelomedusa, Chelone, Caretta*
dorsaler Innenrand:	*Emys, Hardella, Testudo, Emydura*
Jugale:	
Innenfläche: (vorwiegend dors. Bezirk)	*Pelomedusa, Chelone, Caretta*
dorsaler Innenrand:	*Clemmys, Emys, Hardella, Testudo*
Squamosum:	
Innenfläche: (vorwiegend dors. Bezirk)	*Pelomedusa, Testudo, Pelusios, Chelone, Caretta*
dorsaler Innenrand:	*Emys, Hardella, Clemmys*
Quadratum:	
*) Temporalfläche:	*Macrochelys, Emys, Hardella, Testudo, Chelodina, Hydromedusa, Emydura, Graptemys, Trionychiden,*

* Unter Temporalfläche des Quadratum verstehe ich die der oberen Temporalgrube zugekehrten Fläche, die der unteren Temporalgrube zugekehrte Fläche bezeichne ich als Rostralfläche.

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	Pelomedusa, Pelusios, Chelone, Caretta, Podocnemis, Platysternon
Rostralfläche:	*Trionychiden*
Supratemporale:	
Temporalfläche:	*Macrochelys, Pelomedusa, Emys, Hardella, Testudo, Hydromedusa, Graptemys, Platysternon, Trionychiden, Chelone, Caretta, Podocnemis, Emydura, Clemmys, Chelodina*
Caudal- und Ventralfläche:	*Chelodina, Emydura, Hydromedusa*
Prooticum:	
Temporalfläche:	*Pelomedusa, Emydura*
Opisthoticum:	
Temporalfläche:	*Chelodina, Hydromedusa*
caudo-lateraler Fortsatz:	*Trionychiden, Podocnemis*
Caudalfläche:	*Emydura, Testudo elephantopus*
Basisphenoid:	
Caudalfläche:	*Emydura*
Temporalfascie:	
Unterfläche des rostralen Drittels:	*Clemmys*
rostro-laterale Ecke:	*Emys, Hardella, Testudo, Pelomedusa, Pelusios*
lateraler Streifen:	*Emydura*

Ähnlich wie P.N. fand ich, daß nicht nur die Masse der tieferen Fasern, sondern die Masse der Muskelfasern überhaupt an der mächtig ausgebildeten in ihrer Form und Lage außerordentlich variablen Binnensehne ansetzt, während die äußeren Fasern fleischig an der Lateralfläche, weniger an der Dorsalfläche der Mandibel, wie P.N. schreibt, inserieren.

P.N. schreibt u.a., daß die Muskelfasern bei *Caretta* an der Lateralfläche und außerdem noch an einem kräftigen niedrigen, von der Lateralfläche abgehenden Kamm, doppelfiedrig ansetzen. Die Adductorensehne meines Präparates von *Caretta* besitzt zwar eine deutliche Facettierung und Zipfelung, jedoch keine von der Lateralfläche abgehenden Fasern. Was das Einstrahlen der rostralsten oberflächlichsten Superficialisfasern in den Bug der Mundplatte anbelangt, so stimme ich P.N. prinzipiell zu. Jedoch fand ich keine Insertion an der Mundplatte bei *Chelodina*. Bei Pelusios waren es längst nicht sämtliche oberflächliche Fasern, die an der Mundplatte ansetzen. Ansätze von

oberflächlichen rostralen Muskelfasern an Mundplatten gehören bei meinen Präparaten zu den Seltenheiten. Meist ist die Muskelfascie über den rostralsten Fasern der P.superficialis so eng mit der Mundplatte verbunden, daß eine Insertion vorgetäuscht wird. Bei den Schildkröten mit orbitaler Pterygoideusrinne ist die innere Mundplatte an die Adductorensehne angeheftet. Aber auch bei den anderen Schildkrötenformen steht die Adductorensehne häufig in direktem Kontakt zur inneren Mundplatte und zur Mundtasche.

Auch hier gebe ich nachstehend eine übersichtliche Zusammenstellung meiner Insertionsbefunde zur P.superficialis.

Ansatz der P.superficialis

**) Adductorensehne:*	
Außenfläche:	*Macrochelys, Emys, Hardella, Testudo, Graptemys, Trionychiden, Pelomedusa, Chelodina, Podocnemis*
Rücken:	*Platysternon, Graptemys, Emydura*
Ventralfläche:	*Pelomedusa, Emydura, Podocnemis, Pelusios*
lateraler Rand:	*Pelomedusa, Chelone, Caretta, Pelusios*
caudaler Rand:	*Trionychiden, Chelodina*
Supraangulare:	
Lateralfläche:	*Macrochelys, Clemmys, Chelone, Caretta, Pelusios, Testudo, Testudo elephantopus*
Coronoid:	
Lateralfläche:	*Platysternon, Emys, Hardella, Testudo, Pelomedusa, Macrochelys, Chelone, Caretta*
Lateral- und Caudalfläche:	*Trionychiden, Pelusios, Testudo elephantopus*
Mundplatte:	
innere M.P.:	*Macrochelys, Clemmys, Trionychiden* (vereinzelt)
Umschlagstelle:	*Clemmys*
Dentale:	
Lateralfläche:	*Platysternon*

Über die P.superficialis bei *Amyda* schreibt P.N., daß die vom Allgemeintypus stark abweichende sehr breite aber nicht kräftige Portion an der Temporalfascie entspringe, medial vom Jugale und Quadrato-Jugale, und daß der Muskel eine Sehne umschließe, deren Rostral- und Caudalfläche zahlreichen Fasern zum Ursprung diene. Die Sehne sei nicht mit der Binnensehne iden-

* Die Adductorensehne inseriert in der Regel auf der Dorsal-, Medial- und Lateralseite des Proc.coronoides mandibulae (Dentale, Supraangulare, Coronoid, Goniale).

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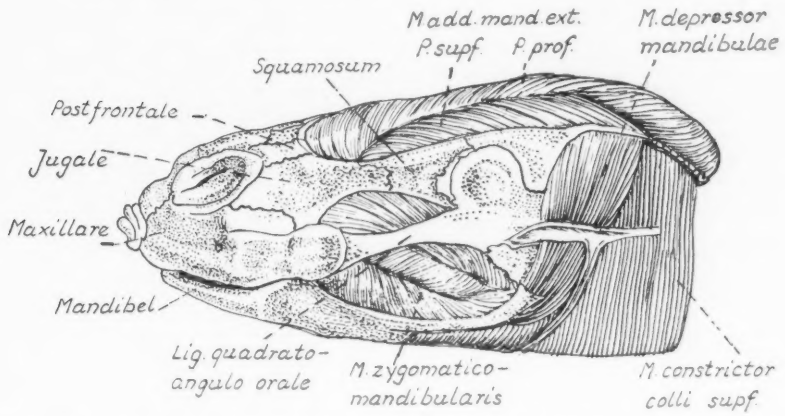

Abb. 3. Kopf von *Trionyx punctatus* (BONNATERRE) bei lateraler Ansicht. Das in situ belassene Lig.quadrato-angulo-orale bedeckt den M.zygomatico-mandibularis.

tisch, sondern eine eigene Differenzierung der P.superficialis. Diese hier von P.N. angegebene Muskelportion rechne ich, wie einleitend ausgeführt, nicht zum M.add.mand.ext., da ein morphologischer Zusammenhang mit dieser fehlt. Über die Lage der P.superficialis schrieb P.N., daß sie die untere Schläfengrube nahezu ausfülle. Die Muskelportion liegt aber nicht in der unteren Temporalgrube. Der von P.N. auf S.246 gegebenen Abbildung E *(Amyda sinensis)* kann ich nicht zustimmen. Dieser Muskel (M.masseter OGUSHI 1926, atypische P.superficialis POGLAYEN-NEUWALL 1953, M.zygomatico-mandibularis (SCHUMACHER 1954) bedeckt das laterale Temporalfenster und liegt unmittelbar medial vom Lig.quadrato-angulo-orale. Nach rostral wird dieser Muskel durch das Maxillare, oben durch den Jochbogen, nach caudal durch den Außenrand des Quadratum und den Kiefergelenkspalt, ventralwärts durch den hinteren Anteil des Unterkiefers begrenzt. Er ist von trapezförmiger Gestalt und verbindet den unteren Rand des Jochbogens mit dem äußeren Rand der Mandibel, wonach ich ihn auch benannt habe. Der vordere Muskelrand erreicht die Hornlamelle des Ober- und Unterkiefers. Während er bei *Trionyx punctatus* zwei Bäuche erkennen läßt, sind dieselben bei *Amyda sinensis* nur angedeutet, der Muskel ist hier im ganzen platter. Ein kleinerer Bauch nimmt den oberen hinteren Teil des lateralen Temporalfensters ein und hat eine mehr spindelige Form. Er liegt unmittelbar unter dem Jochbogen und zieht von der Ecke des Kiefergelenkes nach rostro-dorsal, so daß seine Längsachse eine schräge rostro-caudale Richtung einnimmt, deren rostralster Punkt dorsaler und deren caudalster Punkt ventraler gelegen ist. Die vom Jochbogen entspringenden Fasern ziehen von rostral-dorsal nach caudal-ventral fast parallel zum Jochbogen. Die rostralsten Fasern, welche von einem äußerlich als feinem Streifen sichtbaren Sehnenblatt ihren Ursprung nehmen, verlaufen in horizontaler Rich-

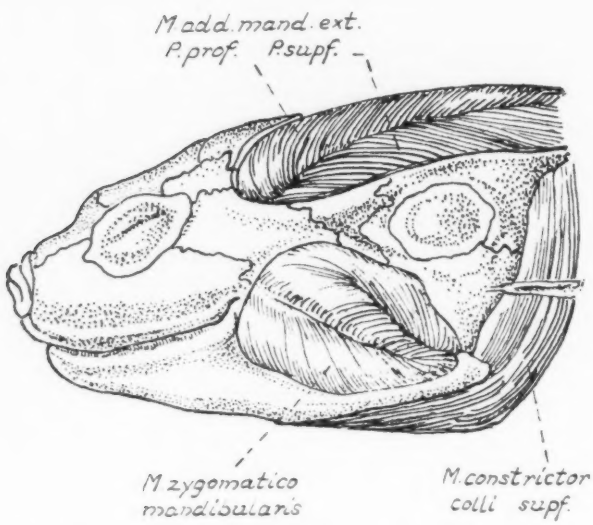

Abb. 4. Wie Abb. 3. Das Lig.quadrato-angulo-orale ist entfernt.

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tung von rostral nach caudal. Alle Fasern inserieren an einer zweiten in der Tiefe des Muskels gelegenen Sehne, welche von der rostralen bzw. lateralen Lippe des breiten Gelenkplateaus des Unterkiefers entspringt und mit der Muskelportion nach rostro-dorsal ansteigt, um unterhalb des knöchernen Jochbogens frei auslaufend zu enden. Nach Art einer Blattrippe nimmt sie alle Fasern des dorso-caudal gelegenen Muskelbauches auf. Der zweite etwas größere Muskelbauch bzw. Muskelanteil nimmt die unteren vorderen ⅔ des lateralen Temporalfensters ein. Mit seiner rostralen Spitze gewinnt er dorsalwärts Anschluß an die Caudalkante des Maxillare, ist rostral von der inneren Mundplatte begrenzt und legt sich unten, d.h. ventralwärts dem Unterkiefer außen auf. Beide Muskelbäuche sind in der Tiefe nicht zu trennen. Die äußerlich trennende Furche ist durch eine Sehne bedingt, welche von lateral nur als feiner Streifen sichtbar wird. Wir haben also zwei Sehnenblätter, ein rostrales und ein caudales. Das caudale ist in der Abb.E von P.N. nicht eingezeichnet. Das erstere ist das stärkere von beiden. Äußerlich imponiert es nur als Streifen und bedingt die Unterteilung des M.zygomatico-mandibularis in zwei Bäuche. Es entspringt vom Knochen des rostro-dorsalen Winkels der lateralen Temporalöffnung und zwar von einem kleinen Knochenhöckerchen an der Ventralseite des Jugale, unmittelbar hinter der inneren Mundplatte. Der Sehnenstreifen zieht nach caudo-ventral, dient einerseits den erwähnten dorso-caudal gelegenen Muskelfasern zum Ursprung und andererseits den ventral befindlichen Fasern, welche im rostralen Bereich außerordentlich kompakt sind, zum Ansatz. Die Sehne läuft in der Muskelmasse frei aus. Ihre Spitze liegt unmittelbar vor dem

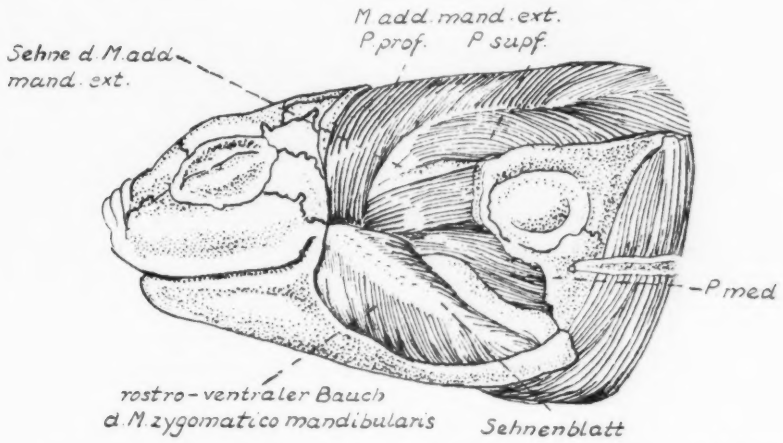

Abb. 5. Das gleiche Präparat wie Abb. 3 nach Resektion des Jochbogens und Entfernen des dorso-caudalen Faseranteils des M.zygomatico-mandibularis.

Kiefergelenkspalt. Die Muskelfasern stellen also eine Verbindung zwischen dem Sehnenblatt und dem Außenrande des Unterkiefers her. Nach caudal verlieren die Fasern ihre Vertikalstellung, hier ziehen sie von rostral dorsal nach ventral caudal. Der Unterkiefer bildet lateral eine Mulde, die nach caudal durch die plateauartige Facies articularis mandibulae einen Abschluß findet. In diese Mulde fügt sich der Muskel ein.

Die Faseranordnung der rostro-ventralen Portion ist derart, daß die lateralsten Fasern am weitesten ventral vom Unterkiefer entspringen und an der nach ventral geneigten Kante des Sehnenblattes inserieren. Die tieferen Fasern verschieben ihren Ursprung immer weiter nach dorsal und erreichen die schräggestellte diagonal verlaufende Sehne an ihrem gehobenen medialen Rande. Aus diesen Insertionsverhältnissen geht hervor, daß die Sehne kein dünner Streifen ist, sondern eine in die Tiefe versenkte Fläche besitzt. Sie hat die Form eines langen spitzen Blattes, ihr Außenrand ist lateral gesenkt, ihr medialer Rand steht höher. Infolge dieser Schrägstellung sind alle Muskelfasern in einer Frontalebene gleich lang, außerdem ist für Ursprung und Ansatz genügend Platz vorhanden.

3. Zur Portio medialis

Von der „P.media" schreibt P.N., daß sie sich in ihrer Stärke umgekehrt zu der P.superficialis verhielte und daß sie bei *Pelusios, Pseudemys, Chrysemys* und *Dermochelys* besonders kräftig sei, bei *Cuora* und *Caretta* dagegen schwach sei und daß sie sich in der Wahl ihres Ursprungs- bzw. Ansatzgebietes recht variabel verhalte. Der Muskel entspränge in der Regel medio-dorsal am Squamosum und am Quadratum medial der P.superficialis. Als zusätzliches Ur-

sprungsgebiet komme bei *Emys, Chelodina, Pelusios* und *Amyda* die Dorsalfläche des Opisthoticum, bei *Caretta, Dermochelys, Terrapene* die Medialfläche des Quadratojugale hinzu. Bei *Chelodina, Kinosternon, Batrachemys* und *Pelusios* zögen die Muskelfasern, einen Ballen bildend, der bei letzterer die P. profunda caudal noch überragt, erst nach dorsal und bögen dann nach rostro-ventral um.

Auch hier ist eine Gegenüberstellung der Angaben beider Autoren infolge der verschiedenen Auffassung über die Ausbreitung dieser Portion erschwert. Die P.medialis entspringt von der Rostralfläche des Quadratum und zwar von der lateralen Hälfte. In wenigen Fällen *(Trionychiden, Pelomedusa)* erfolgt der Ursprung noch von der Kapsel des Kiefergelenkes. Den von P.N. angegebenen dorso-caudalen Faseranteil habe ich bei der ohnehin unscharfen Trennung nicht mehr zur P.medialis gerechnet. Eine Ausnahme in der Abgrenzung zur P.superficialis machen die *Trionychiden*, speziell *Trionyx punctatus*. Beide Portionen sind hier durch einen tiefen horizontalen Spalt voneinander abgesetzt. In der Tiefe ist die Trennung aber auch hier nicht vollständig. Kurz vor ihrer Insertion wird die ventral gelegene P.medialis von einem schmalen Muskelzug überkreuzt, der durch die Sehnenauftreibung der Cartilago transiliens von der Masse der P.superficialis abgesprengt ist. Durch diese Überkreuzung kommt ihre mediale Lage zur P.superficialis zur Geltung, wie es andeutungsweise auch bei *Caretta* der Fall ist. Ihre ventralsten Fasern verlaufen nicht wie üblich horizontal von caudal nach rostral, sie sind caudal etwas tiefer gelegen und steigen infolge des stark ausgebildeten Proc.coronoides mandibulae nach rostral etwas an. Nach medial scheinen die Fasern kürzer zu werden und sich mit den Fasern der P.superficialis vereinigend, nehmen sie allmählich wieder eine Vertikalstellung ein. Nunmehr von dorso-caudal nach rostro-ventral ziehend, erreichen sie den Vorderrand des außerordentlich breiten Gelenkplateaus, an dessen seitlicher Lippe sie inserieren. Ein seitlicher Ausleger des Gelenkplateaus dient gleichzeitig den längeren Muskelfasern, die einen mäßigen Bauch bilden, zum Lager.

Wie man in der nachstehenden Zusammenstellung überblickt, lokalisiert sich der Ansatz der P.medialis in der Hauptsache auf die Lateralfläche und den caudalen Rand der Adductorensehne. Aber auch ein nicht unerheblicher Teil der Muskelfasern inseriert an der Lateralfläche des Proc.coronoides mandibulae.

Ansatz der P.medialis

Adductorensehne:	
Lateralfläche:	*Platysternon, Clemmys, Graptemys, Pelomedusa, Hydromedusa, Chelone, Caretta, Podocnemis, Pelusios*
caudaler Rand:	*Macrochelys, Platysternon, Clemmys, Emys, Hardella, Testudo, Graptemys, Hydromedusa, Chelone, Caretta,*

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	Podocnemis, Pelusios
Unterfläche:	*Platysternon*
Articulare:	
vordere und seitliche Lippe des Gelenkplateaus:	*Trionychiden*
Supraangulare:	
Lateralfläche:	*Macrochelys, Clemmys, Graptemys, Emydura, Emys, Hardella, Testudo, Chelodina, Hydromedusa, Chelone, Caretta, Podocnemis, Pelusios*
Dorsalseite:	*Trionychiden*
Coronoid:	
Lateralfläche:	*Emys, Hardella, Testudo, Pelomedusa, Trionychiden*
Dorsalseite:	*Trionychiden, Emydura*
Dentale:	
Lateralfläche:	*Emys, Hardella, Testudo*
dorso-caudaler Rand:	*Trionychiden*

4. Zur Portio profunda

P.N.'s und meine Auffassung über die Abgrenzung der P.profunda sind wohl nahezu gleich. P.N. schreibt, daß sie durch ihre Lage medial der Binnensehne gegen die beiden anderen Portionen deutlich abgegrenzt sei. Der für die P.profunda typische caudale Muskelwulst kann sehr verschieden stark ausgebildet sein, überragt er die Spina supraoccipitalis so liegt er damit außerhalb der oberen Temporalgrube. *Platysternon megacephalum* macht eine Ausnahme, hier verschwindet der caudale Wulst unter der maximalen Schläfenbedachung in der oberen Temporalgrube. Gewöhnlich ist die P.profunda caudal vom M.splenius capitis bedeckt, der im allgemeinen eine mehr oder weniger tiefe Impression auf diesem Muskel hinterläßt. Sie stellt mit wenigen Ausnahmen die stärkste Portion dar, jedoch nicht durchweg wie P.N. schreibt.

Bei *Platysternon* und *Pelomedusa* ist die P.superficialis etwas größer als die P.profunda. Bei *Emydura krefftii* findet man zwar keine vollkommene Trennung in einzelne Portionen, jedoch ist der caudale Wulst der P.superficialis stärker als der der P.profunda. Das gleiche gilt für *Chelodina*. Auch bei *Pelusios* überragt der caudale Wulst der P.superficialis den der P.profunda. Die Faserverlaufsrichtung ist im allgemeinen bei der einfachen P.profunda folgende: von medial caudal meist etwas dorsal nach rostral lateral und etwas ventral. Der allergrößte Teil der Mittelfasern inseriert an der Adductorensehne, nur in Ausnahmefällen erreicht ein ganz geringer Anteil den Unterkiefer

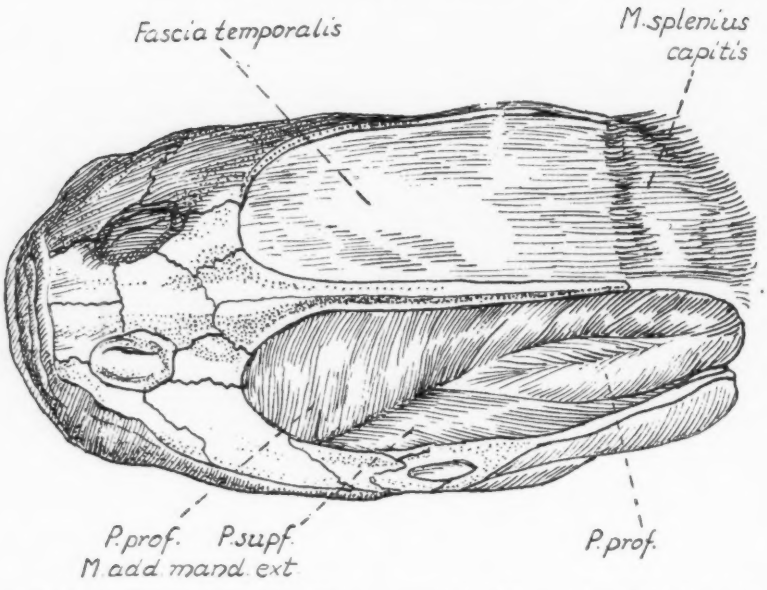

Abb. 6. Kopf von *Trionyx punctatus* (BONNATERRE), Ansicht von dorsal. Links ist der M. adductor mandibularis externus dargestellt. Zu beobachten ist die komplizierte Faserstruktur der P.profunda.

direkt. Bei *Chelodina longicollis* ist die P.profunda gar nicht so schwach, wie P.N. berichtet. Er läßt sie nur vom Supraoccipitale und Parietale entspringen, ich fand jedoch den Ursprung wesentlich weiter ausgebreitet. Daß die Fasern an der Gaumenhaut medial der Mundplatte und an der Dorsalfläche, der teilweise in die Gaumenhaut einstrahlenden Binnensehne ansetzen, habe ich nicht feststellen können. Obgleich die innere Mundplatte mittels Bindegewebe außerordentlich eng mit den Muskelfasern verbunden ist, dient sie keinen Fasern zur Insertion. Einen von P.N. bei *Caretta* angegebenen Ursprung der P.profunda am Boden der oberen Temporalgrube fand ich bei meinen Untersuchungen nicht. Auch bei *Amyda* habe ich den Ursprung weitläufiger gefunden als an der Unterfläche des Postfrontale, am Parietale und Supraoccipitale. Die für die *Trionychiden* so typischen Sehnenblätter finden bei P.N. keine nähere Erklärung. Sie sind jedoch für die Struktur der P.profunda von großer Bedeutung. Hierdurch erhält die Muskelportion eine komplizierte innere Struktur mittels der sie ihre Kraftentfaltung wesentlich verstärkt. Ein gleiches Bauprinzip konnte ich auch bei *Podocnemis expansa* und *Graptemys geographica* finden. Supraoccipitale Sehnenblätter, die dem Ursprung von Muskelfasern dienen, werden in wechselnder Zahl überall dort vorhanden sein, wo es eine langausgezogene Crista supraoccipitalis gibt. Die Architektur, bzw. der Faserverlauf, der P.profunda ist folgende:

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KRITISCHE STELLUNGNAHME ZU DER ARBEIT

Im rostralen Bereiche ziehen sämtliche Muskelfasern in der üblichen Richtung vom Ursprungsareal am Knochen von caudal medial nach rostral lateral und etwas ventral, um an der Medialseite der Adductorensehne zu inserieren. Im caudalen Abschnitt wechselt die Faserverlaufsrichtung durch das Einstrahlen der supraoccipitalen Sehnenblätter. Der von der Crista supraoccipitalis ein- oder zweizipfelig entspringende Sehnenspiegel entsendet beidseitig Muskelfasern. Die medial vom supraoccipitalen Blatt entspringenden Sehnenfasern verlaufen genau in caudo-rostraler Richtung und setzen an der Lateralseite des medialen Zipfels der Adductorensehne an. Die von der Lateralfläche des Supraoccipitalblattes ausgehenden Fasern ziehen von medio-caudal nach rostro-lateral und inserieren an der ganzen Medialseite eines lateralen, sowie auf der Dorsalseite eines ventral gelegenen Ausläufers der Adductorensehne. Über die weiteren Ursprungsareale gibt die nachstehende Übersicht Auskunft. Die P.profunda reicht bis unmittelbar hinter die orbitale Trennwand und liegt der inneren Mundplatte an. In vereinzelten Fällen können auch einzelne Fasern an ihr inserieren. In den meisten Fällen sind sie durch Bindegewebe mit der Mundplatte verbunden. Bei *Clemmys caspica* unterscheidet P.N. zwischen einer laterocaudalen Portion (P.profunda typica), die nicht vom Allgemeintypus der Profundusportion abweicht und einer medio-rostralen Portion (P.profunda atypica). Letztere soll den M.pseudotemporalis des M.add.mand.int. bedecken und am ventro-rostralen Abschnitt des Parietale, genau dorsal der P.pseudotemporalis entspringen und in den ventralsten Teil der Medialfläche der Binnensehne einstrahlen, kurz vor deren Ansatz. Diese Abspaltung soll sich bei *Clemmys caspica* finden, nicht aber bei *Clemmys guttata*. Eine derartige Aufspaltung habe ich weder bei meinem *Clemmys-caspica-rivulata*-Präparat noch bei irgend einer anderen Schildkröte gefunden. Sollte es sich möglicherweise nicht um eine Variation handeln?

	Ursprung der P.profunda
Occipitale:	
Temporalfläche:	*Chelodina, Hydromedusa*
Supraoccipitale:	
Verticalfläche:	*Pelusios, Testudo eleph., Graptemys, Chelodina, Hydromedusa, Emydura, Macrochelys, Platysternon, Trionychiden, Chelone, Caretta, Podocnemis*
Horizontalfläche:	*Macrochelys*
*) Crista supraoccipitalis:	*Clemmys, Emys, Hardella, Testudo, Trionychiden,*

* Der Ursprung von der Crista supraoccipitalis ist größtenteils identisch mit dem der Verticalfläche des Supraoccipitale.

	Pelomedusa, Emydura, Chelone, Caretta, Podocnemis, Pelusios
supraoccipitale	*Graptemys, Trionychiden, Podocnemis*
Sehnenblätter:	
Parietale:	
mediale Wand:	*Graptemys, Platysternon, Clemmys, Emys, Hardella, Testudo, Pelomedusa, Chelodina, Hydromedusa, Trionychiden, Podocnemis, Testudo cleph.* (caud. Drittel)
mediales Parietaldach:	*Platysternon, Emydura*
caudale Kante:	*Clemmys, Emys, Hardella, Testudo*
Proc.descendens:	*Emydura, Chelone, Caretta, Pelusios*
Opisthoticum:	
Temporalfläche:	*Clemmys, Emys, Hardella, Testudo, Graptemys, Macrochelys, Trionychiden, Chelodina, Hydromedusa*
Prooticum:	
Temporalfläche:	*Macrochelys, Trionychiden*
dorsaler caudaler Fortsatz:	*Pelusios*
Postfrontale:	
caudaler Vorsprung:	*Trionychiden, Chelodina, Hydromedusa, Emydura*
Fascia temporalis:	
medialer Rand:	*Emydura, Pelomedusa*
	Ansatz der P.profunda
Adductorensehne:	
Lateralseite:	*Macrochelys, Clemmys, Emys, Hardella, Testudo, Pelomedusa*
lat.Sehnenrand:	*Macrochelys*
Medialseite:	*Macrochelys, Clemmys, Emys, Hardella, Testudo, Graptemys, Podocnemis, Pelusios*
med. Rand:	*Platysternon*
Unterfläche:	*Platysternon, Clemmys, Emys, Hardella, Testudo, Emydura, Podocnemis, Macrochelys*
Dorsalseite:	*Platysternon, Graptemys, Trionychiden* (i.d.unteren Temporalgrube), *Pelomedusa, Emydura, Chelone, Caretta*
dors.Rand:	*Clemmys, Emys, Hardella, Testudo*
med.Sehnenzipfel:	*Trionychiden* (beiderseitig)

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lat.Sehnenzipfel:	*Trionychiden* (Lateralseite)
ventr.Sehnenzipfel:	*Trionychiden* (Dorsalseite)
Mundplatten:	
innere M.P.:	*Trionychiden*
Proc.coronoides mandibulae:	*Trionychiden, Pelomedusa*

b) DER M.ADDUCTOR MANDIBULARIS INTERNUS

Er bekleidet die Wände der unteren Temporalgrube und läßt verschiedene Muskelanteile erkennen, denen infolge ihres Zusammenhanges untereinander der individuelle Charakter fehlt. Durch das Vorhandensein einer gemeinsamen Fascie und einer einheitlichen Sehne wird der Zusammenhang des Muskels noch unterstrichen. Außer der Hüllfunktion begünstigt die Fascie den gegensätzlichen Gleitmechanismus zum M.add.mand.ext.. Sie dient der Führung und Lagerung von Gefäßen und Ästen des N.trigeminus und beteiligt sich am caudalen Weichteilverschluß der Orbita, was besonders bei Schildkröten mit orbitaler Pterygoideusrinne hervortritt. Die Sehne des M.add.mand.int. (kurz Internussehne genannt) ist zum Teil in den Muskel eingebettet oder breitet sich oberflächlich als Spiegel aus. Ihre Form ist recht vielgestaltig und trägt der Lage der einzelnen Muskelabteilungen Rechnung.

Der M.add.mand.posterior breitet sich hinter dem Foramen n. trigemini aus, entspringt von der Rostralfläche des Quadratum, zieht in verticaler Richtung mit konvergierenden Fasern zum caudalen Abschnitt des Unterkiefers, wo er sehnig oder fleischig inseriert.

Der M.parieto-mandibularis verbindet das Parietale mit der Mandibel. Nur bei *Caretta* konnte ich ihn vollständig darstellen, bei *Platysternon* und *Macrochelys* ließ sich der dorsale Kopf von dem darunterliegenden Add. ant. nicht scharf trennen. Die Fasern beider Köpfe inserieren an einer gemeinsamen Zwischensehne, so daß der Muskel die Form eines X oder einer Sanduhr erhält.

Der M.add.mand.anterior bildet das Mittelstück des Internuskomplexes, läßt sich von dem ventral gelegenen Pterygoideus gar nicht und von dem caudal befindlichen Posterior je nach Lage des Foramen n.trigemini nur unvollständig trennen. Rostral grenzt der kräftige Muskelwulst an die Caudalfläche der Membrana obturatoria orbitae.

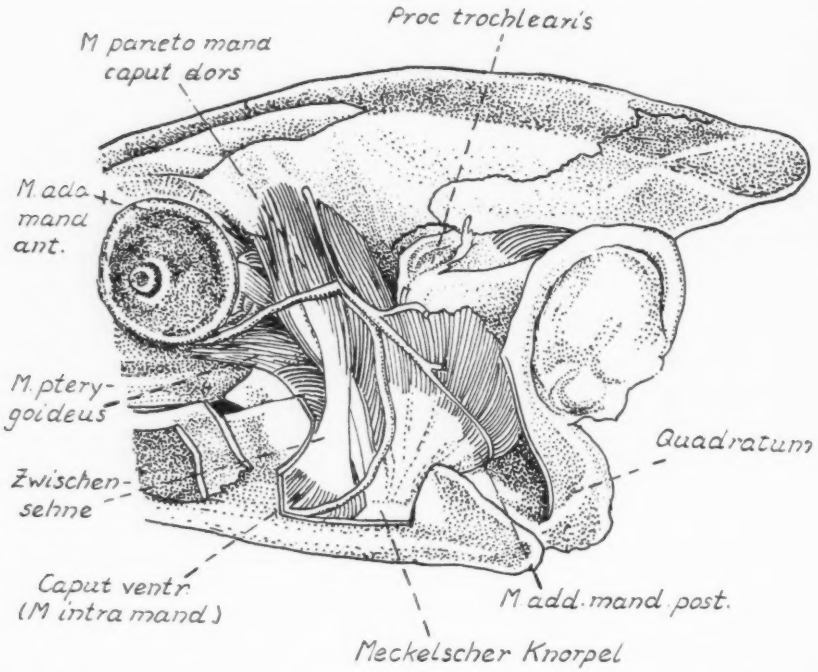

Abb. 7. Einsicht in die linke Temporalgrube von *Caretta caretta* (LINNÉ) nach Resektion des knöchernen Temporaldaches und Teilresektion des Unterkiefers. Zu beachten ist der Komplex des M.adductor mandibularis internus mit dem M.parieto-mandibularis.

Der M.pterygoideus stellt das ventrale Drittel des M.add.mand.int. dar. Bei manchen Schildkröten gibt es eine vom Pterygoid und den benachbarten Knochen gebildete Rinne, die orbitalwärts verläuft und sich kurz vor dem Eintritt in die Orbita zum Kanal schließt. Ist eine solche Rinne vorhanden, so legt sich der Pterygoideus in diese hinein. Ich habe daher bei der Beschreibung des M.pterygoideus grundsätzlich zwischen Schildkröten mit und ohne Pterygoideusrinne unterschieden.

1. Der M.adductor mandibularis posterior

P.N. schreibt u.a., daß der Posterior bei *Pelusios* eine leichte Tendenz zur Bildung zweier Muskelköpfe zeige. Ähnliche Befunde fand ich auch bei meinen Präparaten vor, jedoch konnte ich nicht nur zwei, sondern sogar drei Muskelköpfe darstellen. Ich habe sie nach ihrer Lage als P.media, P.intermedia und P.lateralis bezeichnet. Ein breiter oberflächlicher, dem Muskel rostral aufliegender Sehnenspiegel, bildet zwei verticale Falten, wodurch die oberflächliche Unterteilung des M.add.mand.post. zustandekommt. Die Form der unteren Temporalgrube bedingt eine bogenförmige Anordnung dieser Muskelportionen.

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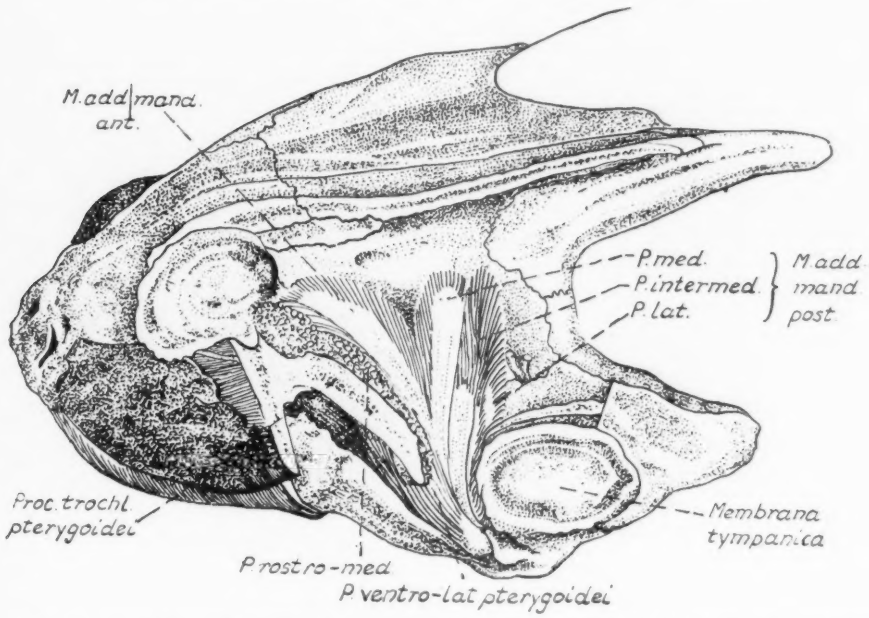

Abb. 8. Dorso-laterale Ansicht des gleichen Präparates wie Abb. 11. Nach Teilresektion des Proc.trochlearis pterygoidei übersieht man den dorsalen Komplex des M.adductor mandibularis internus.

Die P.med. ist die längste und schmalste. Sie liegt unmittelbar hinter dem For.n.trigemini und zieht von dorsal medial nach ventral lateral und bildet den medialen Schenkel des nach rostral offenen Halbkreises. Nach lateral schließt sich, durch die tiefe verticale Falte im Sehnenspiegel scharf abgesetzt, die P. intermedia an. Diese hat eine dreieckförmige Gestalt, deren Basis dorsal liegt und aus den Ursprüngen der Muskelfasern besteht, während die Spitze des Dreiecks den Ansatz medial caudal vom M.pterygoideus am Goniale und Articulare der Mandibel darstellt. Dicht hinter der Ursprungsstelle befindet sich die Apertura canalis carotici. Die P.lateralis faltet sich ebenfalls durch eine Verticalfurche im rostralen oberflächlichen Sehnenspiegel ab. Nur in ihrem medialen und ventralen Abschnitt besitzt sie noch einen kleinen Sehnenspiegel. Ihre Fasern entspringen von der Rostralseite des Quadratum, ziehen in nahezu verticaler Richtung nach ventral und inserieren lateral sowie caudal von der Ansatzstelle des M.pterygoideus, direkt auf der Dorsalseite des Supraangulare, dicht vor dem Articulare. Eine ähnliche oder oberflächliche Unterteilung fand ich auch bei *Podocnemis*. Ich habe auch hier die gleiche Nomenklatur benutzt. Bei manchen Schildkröten war ebenfalls die Andeutung mehrerer Kopfbildungen vorhanden, jedoch habe ich davon abgesehen, weitere Unterteilungen vorzunehmen, da diese Kopfbildungen möglicherweise von der relativen Größe eines Präparates abhängig

sind oder durch die Fixation und der Adductionsstellung des Unterkiefers vorgetäuscht werden können. Auf die vielen kleineren Abweichungen unserer Befunde einzugehen, würde den Leser zu sehr beanspruchen. Das Ursprungsgebiet des M.add.mand.posterior ist ziemlich scharf umgrenzt. Bei allen Schildkröten entspringt er von der medialen Hälfte der Rostralfläche des Quadratum. Bei einem großen Teil erweitert sich der Ursprung bis zum lateralen Drittel der Rostralfläche des Prooticum *(Platysternon, Clemmys, Graptemys, Chelodina, Emydura, Podocnemis, Chelone, Caretta, Pelusios, Testudo elephantopus)*, oder gar bis in die mediale Mulde des Prooticum *(Podocnemis, Pelusios, Testudo elephantopus)*. Selten erreicht der Ursprung noch den Proc. descendens des Parietale *(Pelomedusa, Chelone, Caretta)*.

Ansatz des M.add.mand.posterior

Articulare:	
dorsaler Rand:	*Macrochelys, Podocnemis, Pelusios, Testudo elephantopus*
rostro-medialer Rand:	*Clemmys, Emys, Hardella, Testudo, Graptemys, Pelomedusa, Chelodina, Emydura, Chelone, Caretta, Podocnemis*
Goniale:	
dorsaler Rand:	*Macrochelys, Platysternon, Hydromedusa, Clemmys, Trionychiden, Pelomedusa, Chelodina, Emydura, Chelone, Caretta, Pelusios, Testudo elephantopus*
Medialseite:	*Trionychiden, Chelodina*
Coronoid:	*Trionychiden, Chelodina*
Supraangulare:	
Dorsalseite:	*Chelone, Caretta, Pelusios, Testudo elephantopus, Hydromedusa*
Kiefergelenk:	
Kapsel:	*Pelomedusa*
[1] *Sehne des M.add. mand.int.*	
caudaler Abschnitt:	*Clemmys, Graptemys, Trionychiden, Chelodina, Hydromedusa, Podocnemis*
Fossa primordialis:	
Hinterwand:	*Macrochelys, Emys, Hardella, Testudo, Graptemys*
mediale Wand:	*Hydromedusa*

[1] Man kann den Sehnenspiegel des M.add.mand.post. als Teil der Internussehne auffassen. Die Sehne des M.adductor-internus-Komplexes inseriert an der medialen Seite des Articulare, am dorsalen Rand und an der medialen Seite des Goniale und Coronoid.

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KRITISCHE STELLUNGNAHME ZU DER ARBEIT

Meckel'scher Knorpel:
Dorsalrand: *Pelomedusa*
Lateralseite: *Chelone, Caretta*

2. Der M.parieto-mandibularis

Bei *Caretta* ist es ein verhältnismäßig schwach ausgebildeter schmaler gefiederter Muskel. Vom Caput ventrale, welches P.N. als M.intramandibularis bezeichnet, schreibt er: „Es sei ein gut abgrenzbarer Muskel, der mittels einer Sehne mit der P.pseudotemporalis des Add.internus in Beziehung stehe." Ich fasse ihn nur als Portion meines M.parieto-mandibularis auf. Der dorsale Kopf entspringt vom dorsalen Abschnitt des Proc.descendens des Parietale, zieht in verticaler Richtung ventralwärts. Seine Fasern konvergieren zur Sehne und inserieren an ihr in spitzem Winkel. Die dorsale Portion hat eine dreieckförmige Gestalt, deren Basis dorsal und deren Spitze ventralwärts gerichtet ist. Der ventrale Kopf nimmt seinen Ursprung mit breit ausladender Basis von der lateralen Fläche des Meckel'schen Knorpels. Die Muskelfasern ziehen nach dorsal konvergierend an den unteren Teil der Sehne, die sie kurz vor ihrem Austritt aus der Fossa primordialis erreichen. Die Sehne ist hier etwas breiter und läuft in einem von lateral gut sichtbaren oberflächlichen Sehnenspiegel aus, der sich in seiner Form dem ventralen Muskelkopf gut anpaßt. Diese Portion sieht fächerförmig aus, ihre breite Basis liegt parallel zum Unterrand des Meckel'schen Knorpels, die Spitze zeigt nach dorsal. (s. Abb. 7.)

Um den dorsalen Bauch des M.parieto-mandibularis legt sich der 2.Ast des N.trigeminus, um rostral ziehend in die Orbita einzutreten, dem ventralen Bauch legt sich lateral der Ramus alveolaris nervi trigemini mit der gleichnamigen Arterie und Vene auf. Der Gefäßnervenstrang überkreuzt die P. ventralis in fast rechtem Winkel und zieht von der Fossa primordialis im Canalis mandibularis weiter nach vorn. P.N. hat die P.ventralis bei *Caretta, Pseudemys scripta troostii, Chrysemys picta* und *Graptemys pseudogeographica* gefunden. Seine Benennung „M.intramandibularis" ist recht glücklich gewählt, da sie die Lage dieses Muskelanteiles treffend charakterisiert. Die Sehne (der P.pseudotemporalis) ziehe — so schreibt P.N. — in die Fossa primordialis, wo sie den Fasern eines neuen Muskels, M.intramandibularis der am Boden der Fossa lateral der Cartilago Meckeli[1] ansetze, zum Ursprung diene. Die Sehne inseriere lateral vom Muskel ebenfalls am Boden der Fossa. Ich fand den Ursprung

[1] In einer Fußnote meiner 1.Mitteilung „Beiträge zur Kiefermuskulatur der Schildkröten" bin ich bereits auf die von P.N. gemachten Angaben eingegangen. Herr Poglayen-Neuwall machte mich freundlicherweise auf eine irrtümliche Deutung seiner Angaben über den Ursprung des M.intramandibularis aufmerksam. Ich fand bei meinen Untersuchungen, daß die ventrale Portion von der Lateralfläche des Meckel'schen Knorpels ihren Ursprung nimmt. Da ich P.N. in der Fußnote nicht wörtlich sondern sinngemäß zitierte, schrieb ich irrtümlicherweise: „Und vom M.intramandibularis schreibt er in einem gesonderten Abschnitt auf S.24, daß derselbe bei Caretta *an* der Cartilago ansetze."

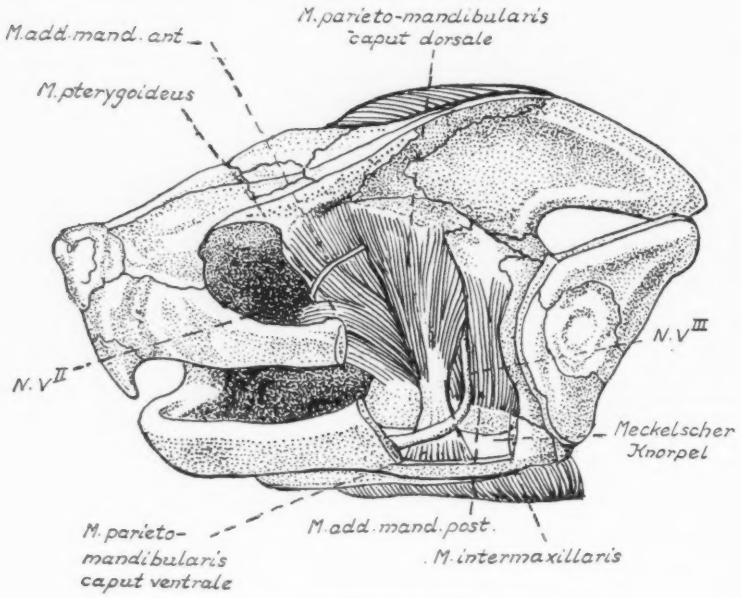

Abb. 9. Wie Abb. 7. Macrochelys temminckii (TROOST).

dieses Muskels nicht auf der Sehne, sondern wie beschrieben, an der Lateralfläche des Meckel'schen Knorpels und den Ansatz nicht am Boden der Fossa primordialis, sondern an der Sehne. Letztere ist das Bindeglied zwischen dorsalem und ventralem Muskelkopf und demzufolge ist eine Insertion derselben am Boden der Fossa, wie P.N. schreibt, nicht erklärlich. Über die Lokalisation des Ursprunges und Ansatzes kann man natürlich geteilter Meinung sein. Ich fasse die Befestigung am Knochen als Ursprung und die Fixation an der Sehne als Ansatz auf. Wir haben also den Typ eines M.biventer bzw. digastricus vor uns, wie wir solche beim Menschen ja auch kennen. Einen Ursprung des dorsalen Kopfes des M.parieto-mandibularis bei Caretta caretta vom Caudalteil des Interorbitalseptum fand ich bei meinem Präparat nicht.

Bei vielen Schildkröten ziehen Muskelteile in die Fossa primordialis hinein. Über das Vorkommen und über das Verhalten derselben kann ich zusammenfassend folgendes aussagen:

1. in manchen Fällen, wie z.B. bei *Caretta, Macrochelys* und *Platysternon* gibt es einen mehr oder weniger klassischen M.parieto-mandibularis. Außer seinem dorsalen Kopf ist er durch eine ventrale, in der Fossa primordialis sich ausbreitende Muskelportion (M.intramandibularis) charakterisiert. Das Zwischenstück am engen Eingang in die Fossa primordialis wird durch eine Sehne gebildet.
2. in anderen Fällen, wie z.B. bei *Emys orbicularis, Hydromedusa tectifera*

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und *Hardella thurjii* ziehen gleichfalls Muskelfasern in die Fossa primordialis hinein, ohne sich jedoch in derselben besonders auszubreiten und ohne an der Eintrittstelle ein sehniges Zwischenstück zu bilden. Hier kann man nicht von einer selbständigen ventralen oder intramandibulären Portion sprechen.

3. gibt es Schildkrötenvertreter, bei denen ein Teil der Sehne des M.add.mand. int. in die Fossa primordialis hineinzieht, (hier ist es häufig die Sehne des M.add.mand.post.) um an der Rück- und medialen Wand der Fossa primordialis oder auch an der Cartilago meckeli zu inserieren. Zu diesen Vertretern gehört z.B. *Graptemys geographica* und *Clemmys caspica rivulata* und
4. gibt es Schildkröten, bei denen weder Muskelfasern noch Teile der Internussehne in die Fossa primordialis gelangen, hierzu gehören z.B. *Emydura krefftii, Trionyx punctatus, Amyda sinensis* und *Dogania subplana.*

Im ersten Falle finden wir einen M.parieto-mandibularis, während wir in den drei letzten aufgeführten Fällen einen solchen vermissen.

3. Der M.add.mand.anterior

Nach P.N. fülle der Add.internus den noch verbliebenen ventro-medialen Raum der unteren Temporalgrube aus, könne sich aber auch wie bei *Caretta* ziemlich weit rostral an das Interorbitalseptum ausdehnen. Daß P.N.'s Muskelteil nie besonders kräftig entwickelt sei, kann ich nicht bestätigen. Im Gegenteil, ich fand meinen M.add.mand.ant. immer relativ kräftig vor, jedenfalls immer stärker als den M.add.mand.post.. Häufig bildet er einen dicken Muskelbauch, der die Form eines Dreiecks besitzt, dessen Basis dorsal, dessen Spitze ventral gerichtet ist. Auch eine Neigung zweiköpfig zu entspringen, habe ich in wenigen Fällen feststellen können. Die Unterteilung in eine laterale und mediale Portion konnte bei mir jedoch zugunsten des M.parieto-mandibularis unterbleiben. P.N.'s laterale Portion ist wohl identisch mit dem dorsalen Kopf meines M.parieto-mandibularis. Eine Zusammenstellung der Ursprünge soll auch hier Details im Text ersparen.

Der Ursprung des M.add.mand.anterior

Parietale:

Proc.descendens: *Macrochelys, Platysternon, Clemmys, Emys, Hardella, Testudo, Graptemys, Trionychiden, Pelomedusa, Chelodina, Hydromedusa, Emydura*

Prooticum:

Rostralfläche: *Macrochelys, Podocnemis, Testudo elephantopus*

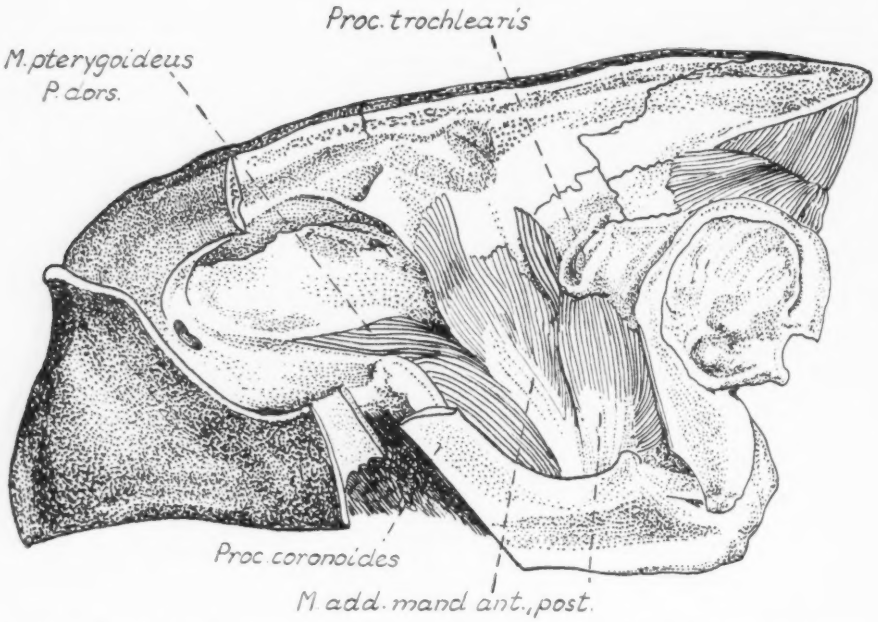

Abb. 10. Das gleiche Präparat wie Abb. 7 nach Ausräumen der Orbita, Entfernen der Nerven und Gefässe. Zu beachten ist der Zusammenhang des M.adductor mandibularis internus.

Postfrontale:
Unterfläche: *Macrochelys, Trionychiden, Hydromedusa, Emydura*

Frontale:
Unterfläche: *Macrochelys, Trionychiden, Hydromedusa, Emydura*

Wie man sieht, ist der Ursprung recht einheitlich. Entweder ist der Muskel lateral von einem sich seiner Form anpassenden Sehnenspiegel bedeckt, an dessen Medialseite die Fasern inserieren, oder es findet sich in der Tiefe seines Bauches ein Sehnenstück, welches den Fasern beidseitig zur Insertion dient. Dieses Sehnenstück steht meist mit dem Sehnenspiegel des Posterior im Zusammenhang, immer aber mit der Sehne des Pterygoideus.

Der Ansatz des M.add.mand.anterior

Coronoid:
Dorsomedialrand: *Macrochelys, Platysternon, Emys, Hardella, Testudo, Graptemys, Clemmys, Chelodina*

Goniale:
Dorsomedialrand: *Macrochelys, Platysternon, Clemmys, Emys, Hardella, Testudo, Pelomedusa, Emydura*

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Articulare:	
Medialseite:	*Macrochelys, Platysternon, Emys, Hardella, Testudo*
Dentale:	
Dorsomedialrand:	*Chelodina*
Meckel'scher Knorpel:	
a) mittels Sehnenausläufers:	
oberer u.lat.Rand:	*Clemmys, Graptemys*
b) fleischig:	
dorsaler Rand:	*Hydromedusa*
Lateralfläche:	*Emys, Hardella, Testudo*
*) *Sehne des M.add. mand.int.:*	*Macrochelys, Platysternon, Clemmys, Emys, Hardella, Testudo, Graptemys, Pelomedusa, Chelodina, Trionychiden*

4. Zum M.pterygoideus

Der M.pterygoideus hatte in meiner 1.Mitteilung mein besonderes Interesse erregt, da er bei *Podocnemis* und *Pelusios* in einer eigenen mit der Orbita kommunizierenden Knochenrinne verläuft und eine besondere Architektur erkennen läßt. Diese Knochenrinne ist bedingt durch einen flügelförmigen walzenartig verdickten Fortsatz des Pterygoidbeines, welcher von medial nach lateral in leicht geschwungenem Bogen horizontal in die untere Temporalgrube hineinragt. Diesen Pterygoidfortsatz habe ich als Proc.trochlearis bezeichnet. Bei meinen weiteren Präparationen fand ich diese orbitale Pterygoideusrinne außer bei *Pelusios* und *Podocnemis* noch bei *Emydura krefftii, Pelomedusa subrufa, Hardella thurjii, Hydromedusa tectifera* und *Chelodina longicollis.* Die von mir untersuchten Trionychiden besitzen keine ausgesprochene Pterygoideusrinne. Es findet sich bei ihnen eine rinnenförmige, orbitalwärts ziehende Vertiefung, in welcher der M.pterygoideus eingebettet liegt.

Eine Portionenunterteilung des M.pterygoideus erschien mir in Anbetracht seiner verschiedenen Ursprungsareale unerläßlich. Die Untergliederung ist zwar bei jüngeren bzw. kleineren Untersuchungsobjekten mit weniger scharf konturiertem Knochenrelief nicht so markant wie bei größeren, aber zweifellos eindeutig. Bei Schildkröten mit orbitaler Pterygoideusrinne bezeichne ich den allseitig aus ihr entspringenden Muskelanteil als P.rostro-medialis. Durch eine laterale Aufbiegung des Proc.trochlearis pterygoidei wird der Eingang zur

* Bei der Vielgestaltigkeit der Internussehne habe ich hier auf nähere Angaben verzichtet.

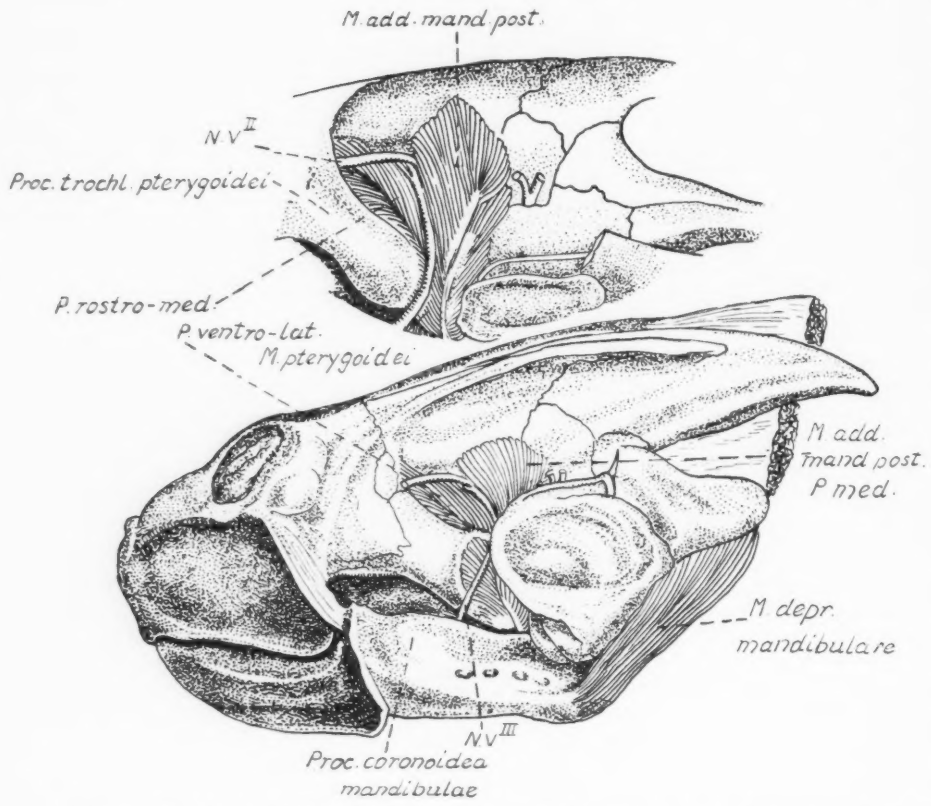

Abb. 11. Einsicht in die linke Temporalgrube von *Podocnemis expansa* (SCHWEIGGER) nach Resektion des knöchernen Temporaldaches. Der M.adductor mandibularis internus ist durch den Proc.trochlearis pterygoidei zum Teil verdeckt. Darüber Teildarstellung bei dorsaler Ansicht.

orbitalen Pterygoideusrinne trichterförmig erweitert und der Fortsatz erhält eine Caudalfläche. Hier entspringt eine zweite Portion, die ich als P.ventro-lateralis beschrieben habe. Nach ventral bildet das Pterygoidbein meist noch einen breitflächigen schaufelartigen Fortsatz, wodurch der Knochentrichter auch nach medial eine Begrenzung erfährt. Bei *Podocnemis* fand ich sogar eine dritte Portion, die ihren Ursprung von der ganzen Innenfläche eines knöchernen Muskelkanales nimmt, der medial vom Quadratum beginnt und in rostro-medialer Richtung sich unter das ganze Pterygoidbein bis zur medialen Scheidewand verfolgen läßt. Ihre Muskelfasern setzen im spitzen Winkel an einer kräftigen Mittelsehne an, welche der Verlaufsrichtung des knöchernen Kanales folgend, denselben verläßt, um am Proc.retroarticularis und am Goniale der Mandibel anzusetzen; z.T. inserieren die aus der trichterförmigen Erweiterung entspringenden Fasern direkt am Proc.articularis. Nur am Austritt aus dem knöchernen

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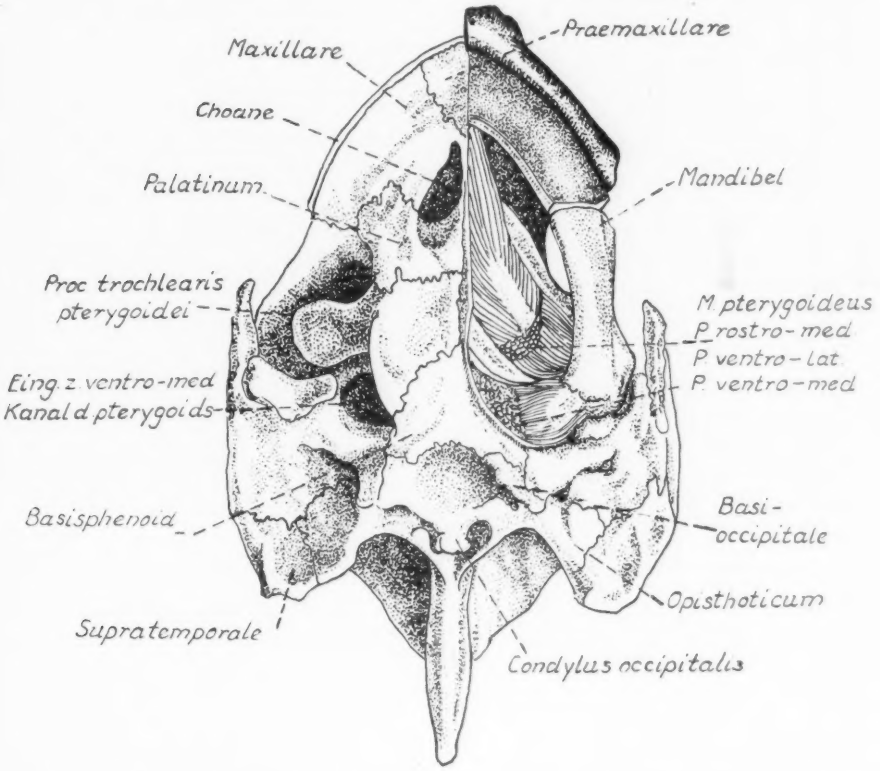

Abb. 12. Das gleiche Präparat wie Abb. 11 von ventral. Nach Resektion des Palatium, Pterygoid und Basisphenoid erkennt man die ventralen Portionen des M.pterygoideus.

Kanal wird die Trennung gegen die P.ventro-lateralis unscharf. Bei dieser Ausnahme läßt der M.pterygoideus als ein Bestandteil des Internuskomplexes keine scharfe Trennung seiner Portionen zu. Bei Schildkröten ohne orbitale Pterygoideusrinne habe ich manchmal dem Ursprunge entsprechend eine P. dorsalis und P.ventralis unterschieden.

Übersicht über die Ursprünge des M.pterygoideus

Pterygoid:

Dorsalseite:	*Platysternon, Emys, Hardella, Testudo, Graptemys, Trionychiden* (P.dors.)
dorsaler Fortsatz:	*Clemmys, Testudo elephantopus* (P.dors.)
rostraler Fortsatz:	
(Dorsalfläche):	*Clemmys, Testudo elephantopus* (P.dors.)
rostraler Fortsatz:	

(Ventralseite):	*Clemmys, Testudo elephantopus* (P.ventr.)
rostraler Fortsatz:	
(lateraler Rand):	*Clemmys, Testudo elephantopus* (P.ventr.)
Ventralseite:	*Platysternon, Emys, Hardella, Testudo, Graptemys, Chelone, Caretta* (P.ventr.) *Pelomedusa, Podocnemis, Pelusios* (P.ventro-lat.)
ventr.Pterygoidfortsatz (med.Fläche):	*Podocnemis, Pelusios* (P.ventro-lat.)
ventro-med.Kanal:	
(ganze Innenfläche):	*Podocnemis* (P.ventro-med.)
freier Rand:	*Trionychiden, Chelone, Caretta*
Caudalfläche:	*Pelomedusa, Emydura, Podocnemis, Pelusios* (P. ventro-lat.)
*) Pterygoideusrinne:	*Clemmys, Emydura, Pelomedusa, Podocnemis, Pelusios, Hardella* (P.rostro-med.) *Chelodina, Hydromedusa*
Palatinum:	
Dorsalseite:	*Platysternon, Clemmys, Emys, Hardella, Testudo, Graptemys* (P.dors.) *Trionychiden*
Ventralseite:	*Platysternon, Emys, Hardella, Testudo, Graptemys,* (P.ventr.) *Pelomedusa* (P.ventro-lat.)
caudaler Fortsatz:	*Chelone, Caretta*
**) *Orbita:*	
caud.Knochenrand:	
am Boden der Orbita:	*Macrochelys*
Boden:	*Platysternon, Chelodina, Chelone, Caretta, Podocnemis, Pelusios*
Parietale:	
Proc.descendens:	
(ventrales Ende):	*Clemmys, Chelodina, Chelone, Caretta, Testudo elephantopus* (P.dors.) *Trionychiden*
Postfrontale:	
Unterfläche:	*Trionychiden*

* Der Ursprung aus der orbitalen Pterygoideusrinne bzw. -kanal erfolgt allseitig und trifft für die P.rostro-medialis zu. An ihrer Bildung sind folgende Knochen beteiligt: medial der Proc.descendens des Parietale, dorsal die Unterfläche des Frontale und Postfrontale, lateral die laterale Wand des Proc.trochlearis pterygoidei, ventral das Pterygoid und Palatinum.

** Bei allen Schildkröten erreicht der Ursprung den Boden der Orbita, es sind jedoch nur solche aufgeführt, bei denen sich der Ursprung weit auf den Boden der Orbita vorschiebt. Hier handelt es sich um die dorsale Portion, in den beiden letzten Fällen um die P.rostro-medialis.

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Jugale:	
caudaler Fortsatz:	*Trionychiden*
Maxillare:	
caudo-lat.Fortsatz:	*Trionychiden*
bdgew.Unterlage der Hornlamelle:	*Chelone, Caretta* (P.ventr.)
Quadratum:	
Ventralfläche: (med.Seite d.Proc.art. oss.quadrati):	*Chelone, Caretta* (P.ventr.)
palatinale mediane Raphe:	*Chelone, Caretta* (P.ventr.)

Wie man sieht, besitzt der M.pterygoideus nicht nur ein recht ausgedehntes, sondern auch recht vielfältiges Ursprungsareal. Daß der M.pterygoideus kräftiger sein soll als der M.add.mand.ant. (M.pseudotemporalis), konnte ich nicht feststellen. Der Anterior ist häufig der dickste und massivste Muskel des Internuskomplexes.

Der Ansatz des Pterygoideus besitzt nicht die Vielfalt wie der Ursprung. Der größte Teil der Muskelfasern inseriert an der Sehne des Muskels, die den rostralen Teil der Internussehne darstellt, der geringere Teil direkt an dem Knochen des Unterkiefers. Eine zusätzliche Insertion an der Gaumenhaut, wie sie P.N. bei einigen Schildkröten angegeben hat *(Kinosternon, Terrapene, Caretta)*, konnte ich nirgends finden.

Zusammenfassung der verschiedenen Insertionen des M.pterygoideus

Internussehne:	
rostraler Sehnenzipfel:	*Macrochelys, Platysternon, Clemmys, Emys, Hardella, Testudo, Graptemys, Trionychiden, Pelomedusa, Chelodina, Hydromedusa, Emydura, Chelone, Caretta, Podocnemis, Pelusios*
Coronoid:	
Dorso-Medialseite:	*Platysternon, Trionychiden,* (am caudalen Abhang) *Pelomedusa*
Supraangulare:	
Dorsalseite:	*Clemmys, Podocnemis, Pelusios, Testudo elephantopus*

Goniale:	
Dorsomedialseite:	*Clemmys, Trionychiden, Pelomedusa, Hydromedusa, Emydura, Chelone, Caretta, Podocnemis, Pelusios, Testudo elephantopus*
Articulare:	
Proc.retroarticularis:	*Clemmys* (Sehnenspiegel) *Chelone, Caretta, Podocnemis*
Dorsomedialseite:	*Clemmys, Emys, Hardella, Testudo, Graptemys, Pelomedusa, Emydura, Chelone, Caretta, Podocnemis, Pelusios*
Medio-ventralfläche:	*Testudo elephantopus*
Angulare:	
Medialseite:	*Clemmys, Emys, Hardella, Testudo, Graptemys*
Kapsel des Kiefergelenkes:	
medialer Mandibularrand:	*Platysternon*

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Zur Sehne des M.add.mand.internus sei bemerkt, daß sie häufig zwei Blätter erkennen läßt. Ein vertical gestelltes nimmt die Fasern des M.add.mand. ant. und kurz vor seinem Ansatz noch die Fasern des M.add.mand.post. auf, es zieht von rostro-dorsal nach ventro-caudal und etwas lateral. Abduziert man den verticalen Sehnenspiegel, so sieht man in der Tiefe eine kleine, nicht so weit nach rostral reichende, mehr horizontal auf dem Pterygoideus gelegene Sehnenplatte. Beide Blätter stehen in einem rechten Winkel zueinander. Letzterer ist das wesentlichste Insertionsgebiet des M.pterygoideus, seine Form und Größe richtet sich ganz nach den Bedürfnissen des Muskels. In seiner Verlaufsrichtung kreuzt er die Sehne des M.add.mand.ext., ist jedoch von dieser durch Muskelmassen und Bindegewebe getrennt.

5. Zum M.intermaxillaris

Diesen Muskel habe ich nur bei *Testudo graeca* zusammen mit G. HACKER präpariert und beschrieben. P.N. bezeichnet ihn als M.intermandibularis. Ich stimme mit den Ergebnissen von P.N. überein. Ergänzend möchte ich zu seinen Befunden noch hinzufügen, daß sich im Mundboden von *Testudo graeca* drei Muskellücken finden, eine rostrale, die wir als Trigonum maxillare rostrale und zwei hintere, die wir als Trigona intermaxillares caudales bezeichnet haben. Die vordere Muskellücke ist von dreieckförmiger Gestalt und wird dadurch gebildet,

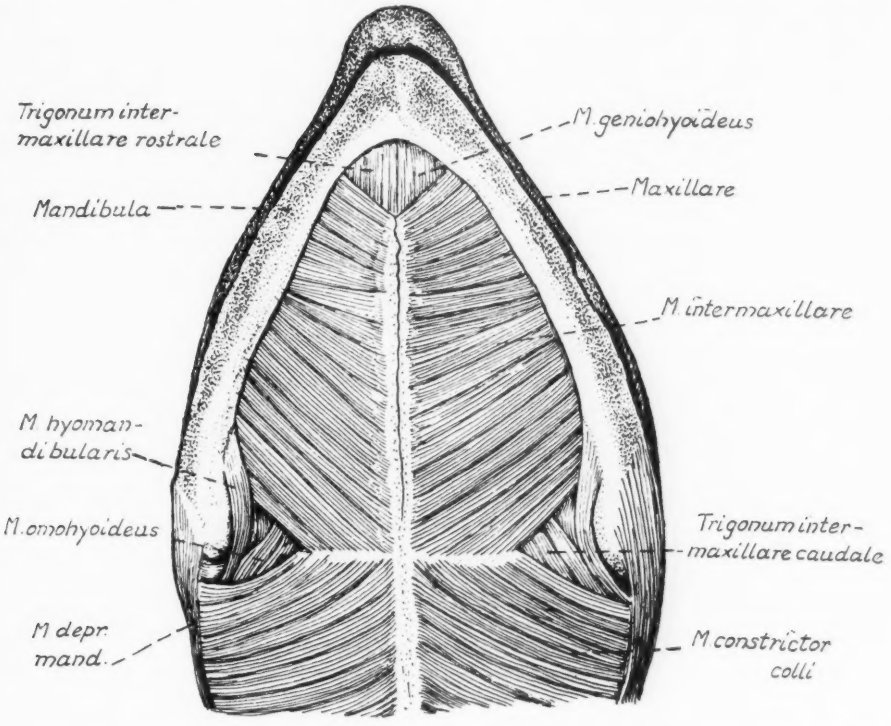

Abb. 13. Ventrale Ansicht eines Kopfes von *Testudo graeca* (LINNÉ) nach Entfernen der Haut und der Fascia subcutanea. Zu beachten sind die Muskellücken.

daß der M.intermaxillaris rostral den Unterkieferbogen nicht ganz erreicht. In der Tiefe dieser Muskellücke wird der rostralste Anteil des M.geniohyoideus sichtbar. Der M.intermaxillaris ist lateral durch die beiden Unterkieferäste begrenzt, caudal findet er seinen Abschluß durch eine quere Raphe, welche gleichzeitig die rostrale Begrenzung für den M.constrictor colli darstellt. Infolge der Faserverlaufsrichtung des M.intermaxillaris sowie des M.constrictor colli kommt es im lateralen caudalen Bereiche des Mundbodens, also in Höhe der Querraphe, wiederum zur Ausbildung einer Muskellücke, welche jedoch paarig ist, in deren Tiefe der M.hyomandibularis und der M.omohyoideus sichtbar werden. Auch sie sind von dreieckiger Form. Der vordere Schenkel des Dreiecks wird durch die caudalsten Fasern des M.intermaxillaris, der hintere Schenkel durch die rostralsten Fasern des M.constrictor colli dargestellt. Die Basis dieser Dreiecke wird durch das Angulare bzw. durch den Proc.retroarticularis mandibulae gebildet.

Auch bei der Präparation des Mundbodens ließen sich Fascien darstellen: Eine Fascia intermaxillaris superficialis als rostro-ventrale Fortsetzung der

Fascia colli supf. im Verein mit der Muskelfascie des M.intermaxillaris, eine aus zwei Blättern bestehende Fascia intermaxillaris medialis und eine profunda, die caudal von den Speicheldrüsen liegt und dieselben von der Zunge trennt.

IV. ZUSAMMENFASSUNG

In der hier vorliegenden Arbeit wurden den Befunden von POGLAYEN-NEUWALL über „Untersuchungen der Kiefermuskulatur und deren Innervation bei Schildkröten" eigene Untersuchungsergebnisse gegenübergestellt.

Die von beiden Verfassern gebrauchte Nomenklatur basierte auf dem von RUGE und LUTHER aufgestellten und von LAKJER-LUBOSCH übernommenen Einteilungsprinzip. Die Ansichten beider Verfasser über die Aufgliederung der Kaumuskeln gehen zum Teil auseinander. Verfasser gliedert die Kiefermuskulatur der Schildkröten folgendermaßen:

Der *M.adductor mandibulae* wurde in einen M.adductor mandibularis *externus* und *internus* unterteilt.

Ersterer läßt drei Portionen erkennen, Portio superficialis, medialis und profunda. Bei den *Trionychiden* wird dem Externus der funktionell mit diesem im Zusammenhang stehende, morphologisch jedoch selbständige M.zygomatico-mandibularis zugeordnet. Der Internus wird als Muskelkomplex aufgefaßt und in einen M.add.mand.posterior, anterior, pterygoideus und in einen bisher nur bei *Caretta, Macrochelys* und *Platysternon* gefundenen M.parieto-mandibularis untergliedert. Allen genannten Muskeln geht ein kurze überblicksmäßige Beschreibung voraus. Es erfolgt eine Gegenüberstellung der von POGLAYEN-NEUWALL erhobenen Befunde unter möglichst intensiver Ausfüllung der sich aus der verschiedenen Thematik und dem Material ergebenden Lücken. Die unterschiedlichen Ergebnisse beider Verfasser resultierten im wesentlichen aus den verschiedenen Ansichten über das dargelegte Einteilungsprinzip. Die Fascienverhältnisse des Kopfes sowie die Binnensehne des Externus und Internus wurden nur berührt. Bei der Erwähnung des M.intermaxillaris wurde auf drei Muskellücken hingewiesen. Die Innervation der Muskulatur wurde nicht untersucht.

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KRITISCHE STELLUNGNAHME ZU DER ARBEIT

V. LITERATURVERZEICHNIS

1. Adams, L. Allen, 1919. A memoir on the Phylogeny of the Jaw Muscles in Recent and Fossil Vertebrates. Ann.New-York, Acad.Sci.Vol.28.New-York.
2. Bojanus, L. L., 1819—21. Anatome Testudinis Europeae, Vilnae.
3. Bolk, Göppert, Kallius, Lubosch, 1938. Handbuch der vergleichenden Anatomie der Wirbeltiere. Bd.IV. u. V. Berlin.
4. Bradley, Charnock, O. 1903. The Muscles of mastication and movement of the skull in Lacertilia. Zool.Jahrb.Abt. für Anat. und Bd.18, Heft 4 pp. 475—486. Jena.
5. Brehm, 1912. Tierleben, 4.Aufl. Lurche und Kriechtiere.Bd.I, Leipzig und Wien.
6. Bronn, H. G. 1890. Klassen und Ordnungen des Tierreichs. Bd.VI,Abt.III, Leipzig u.Heidelberg.
7. Edgeworth, F. H., 1907. The Development of the Headmuscles in Gallus domesticus, and the Morphology of the Headmuscles in the Sauropsida. Quart.Micro.Sci. 51, 511—566, London.
8. —. 1911. On the Morphology of the Cranial Muscles in some Vertebrates. Ibd. 56, 167—316, London.
9. Fritsch, A., 1870. Zur Anatomie der Elephanten-Schildkröte (Testudo elephantina) in Abhandl.d.K.böhm.Ges.d.Wissensch. VI.Folge 4.Bd. Prag.
10. Fuchs, H., 1931. Über den Unterkiefer und die Unterkiefernerven der Arrauschildkröte (Podocnemis expansa). Zeitschr. f.Anatomie und Entwicklungsgesch. Bd.94. Berlin.
11. —. 1915. Über den Bau und die Entwicklung d. Schädels der Chelone imbricata. 1. Teil Voeltzkows Reise in Ostafrika in den Jahren 1900—05. Bd.V, Stuttgart.
12. Gadow, H. 1909. Amphibia and Reptiles. Cambridge Natural History, Vol.8.
13. Gegenbaur, C. 1898. Vergleichende Anatomie der Wirbeltiere mit Berücksichtigung der Wirbellosen 1. Leipzig.
14. Hacker, G. — Schumacher, G. H. 1955. Die Muskeln und Fascien des Mundbodens bei Testudo greaca. Anat.Anz.,Bd. 101, H. 20/24, Jena.
15. Huxley, T. H. 1873. A Manuel of the Anatomy of Vertebrate Animals. London 1871. Dtsch.Übersetzung: Handbuch der Anatomie der Wirbeltiere. von F. Ratzel, Breslau.
16. Lakjer, Tage. 1926. Studien über die trigeminusversorgte Kaumuskulatur der Sauropsiden.Kopenhagen.
17. Lubosch, W. 1913. Die Kaumuskulatur der Amphibien, verglichen mit der der Sauropsiden und Säugetiere. Anat.Anz.Ergänzungsheft Vol.44. Jena.
18. —. 1933. Untersuchungen über die Visceralmuskulatur der Sauropsiden. Morph.Jahrb. 72.Bd. 584—666. Leipzig.
19. Luther, A. 1914. Über die vom N.Trigeminus versorgte Muskulatur der Amphibien. ibidem.
20. Meckel, I. F. 1829. System der vergleichenden Anatomie. 2.Theil, 3.Theil, Halle.
21. Ogushi, K. 1913. Anatomische Studien an der japanischen dreikralligen Lippenschildkröte (Trionyx japonicus) II.Mitteilung. Muskel- und peripheres Nervensystem. Morph.Jahrb.Bd.46, 299—562, Leipzig.
22. Poglayen-Neuwall, Ivo. 1953/54. Die Besonderheiten der Kiefermuskulatur von Dermochelys coriacea. Anat.Anz.,Bd. 100, H. 1/4. Jena.
23. —. 1953. Untersuchungen der Kiefermuskulatur und deren Innervation bei Schildkröten. Acta Zoologica, Arg.34, Häft 3. Stockholm.
24. Pope, G. H. 1949. Turtles of the United States & Canada, New York.

25. SCHUMACHER, G. H. 1953/54. Beiträge zur Kiefermuskulatur der Schildkröten. 1.Mitteilung, Wiss.Zeitschr.d.Univ.Greifswald,Jahrg.III, math.-nat.Reihe Nr. 6/7, 457—518.

26. —. 1954/55. Beiträge zur Kiefermuskulatur der Schildkröten. 2.Mitteilung, Wiss.Zeitschr. d.Univ.Greifswald,Jahrg.IV, math.-nat.Reihe Nr. 5, 501—523.

27. —. 1954/55. Beiträge zur Kiefermuskulatur der Schildkröten. 3. Mitteilung, Wiss. Zeitschr.d.Univ.Greifswald,Jahrg.IV, math.-nat.Reihe Nr. 6/7, 560—601.

28. —. 1956. Über Fascien des Kopfes der Schildkröten nebst einigen Bemerkungen zu der Arbeit von Tage Lakjer 1926. Zool.Anz. Bd. 156 H. 3/4, Leipzig.

29. — . 1956. Morphologische Studie zum Gleitmechanismus des M.adductor mandibularis externus bei Schildkröten. Anat.Anz. Bd. 103 H. 1/4, Jena.

30. SIEBENROCK, F. 1897. Das Kopfskelett der Schildkröten. Sitzungsberichte der Kaiserlichen Akademie d.Wissenschaften. Bd. 106, Abt.I, 245—328. Wien.

31. —. 1909. Synopsis der rezenten Schildkröten, mit Berücksichtigung der in historischer Zeit ausgestorbenen Arten. Zool.Jahrb., Heft 3, Supplement. Jena.

32. VERSLUYS, J. 1936. Kranium und Visceralskelett der Sauropsiden, I.Reptilien. Handb.d. vergl.Anat.d.Wirbeltiere 6. 1936 herausgeg. von Bolk, Göppert, Kallius, Lubosch. Berlin und Wien.

33. WIEDEMANN, CRW. 1802/03. Anatomische Beschreibung der Schildkröten. Arch.Zoologie Zootomie. Vol. 2, St.2 177—210 u.St.3,St.2, 78—102. Braunschweig.

34. ZDANSKY, O. 1924. Über die Temporalregion der Schildkröten. Bull.Geol.Inst.Upsala, Vol. XIX.

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ON THE BODY TEMPERATURE OF TETRAO UROGALLUS LIN. AT HATCHING, AND ITS IMMEDIATELY FOLLOWING DROP

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The body temperature of birds has been the object of close studies. No information is, however, available concerning the body temperature at the very moment the young leaves the shell. Therefore, on lines similar to those followed by HÖGLUND (1955) we have measured this temperature with the intention of analyzing the cause of the low temperature some minutes after hatching, observed by several authors (cf. HÖGLUND, 1955). This research has been performed on the capercaillie, *Tetrao urogallus* Lin., at the research station of the Swedish Hunting Commission at Gullgruva, and the experiments have been planned with the supposition that the drop in body temperature during the drying period is caused to some degree by the evaporation from the wet chickens. Therefore it has been necessary to perform the experiments at a constant air temperature but with varying humidity. In these experiments 45 nestlings of the capercaillie have been used. The eggs obtained from wild birds were gathered in Hälsingland, Jämtland, Norrbotten and Lapland, and belonged to 11 broods. Generally the eggs were brooded 2—3 weeks by the hen capercaillie before placed under a domestic hen in the field. They were then transported by car or train under this sitting hen to Gullgruva, where they were placed in an incubator at a temperature of 37° C.

Towards the end of the incubation when the first fissure in the shell has appeared, the eggs are transferred from the incubator to a specially constructed work-thermostate (Fig. 1) in which both temperature and humidity can be varied. The heating is accomplished by a dark radioator, its effect being regulated with a contact-thermometer and a Hg-relay. The circulation of the air is brought about by a screw, mounted in the opening of a drum, through which the whole air volume of the thermostate is sucked. The circulation therefore is efficient without excessive air velocities, and its speed may be varied within wide limits by regulating the speed of the screw. In order to secure an even distribution of the heat the radiator is placed within the drum, where it is shielded off by a circular metal disc in the opening of the drum. In this way the nestlings will not be exposed to any direct radiation from the heater. Both

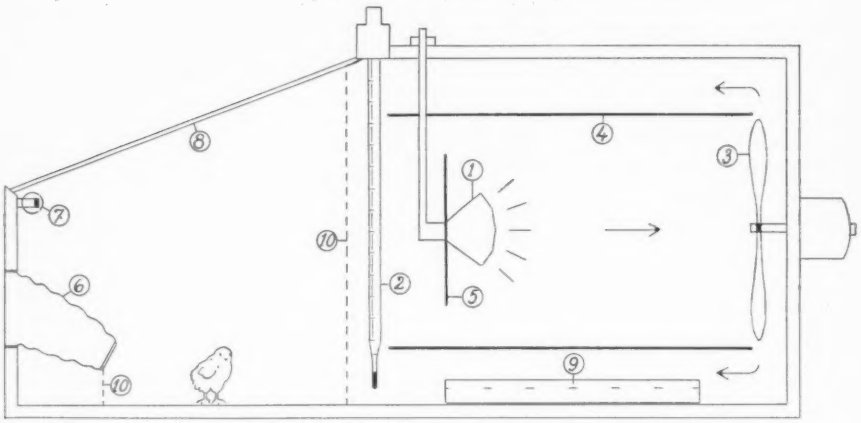

Fig. 1. Section of work-thermostate. 1 radiator, 2 contact thermometer, 3 screw, 4 drum, 5 metal disc, 6 cuff, 7 lamp, 8 double pane, 9 container with water, 10 grating.

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apparatus and birds may be easily handled through two cuffs on the front of the thermostate. The thermostate is illuminated by a tubular lamp, the luminosity of which can be regulated with a resistance. Through a large double pane on the top of the front part it is easy to follow the different phases of the hatching.

Low degrees of humidity are obtained by exposing a water surface of suitable size to the circulating air in the thermostate. By supplying the drum with vapour from boiling water, saturation is very nearly reached. Although the pane is double with an air layer between the glasses the temperature of its inner surface is somewhat lower than that of the air enclosed in the thermostate. This causes a condensation of water at the pane, thus creating difficulties in keeping the air quite saturated with water vapour.

A quick measurement of the body temperature was performed with a thermo-electrical devise, Cuttiterm, type CTTI. This instrument consists of two main parts, a probe and a sensitive spotlight galvanometer. The probe is made up by a copper-constantan thermo-couple, mounted at the end of a 42 mm long needle, on a handle of insulating material. The needle has a diameter of about 1 mm.

When measuring the temperature the probe with its cable was introduced by one of the cuffs into the thermostate. During the experiments one person was occupied with the instrument outside the thermostate and another managed the probe. The thermo-couple was inserted for about 22 mm into the rectum, and the temperature red to within 0.1° C. The greatest caution was necessary in order to prevent damage of the mucous membrane of the rectum.

During the whole series of experiments the speed of air circulation in the thermostate was kept constant. The temperature in the thermostate was 37.3

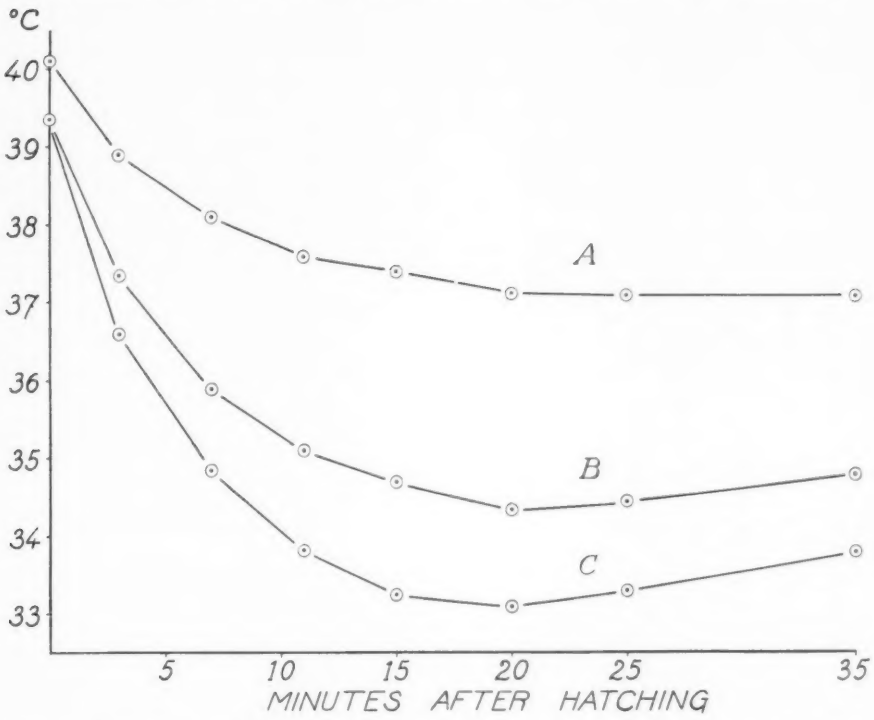

Fig. 2. Mean values of body temperature against time. A in 100, B in 67.5 ± 2.5, and C in 55 ± 2.5 per cent relative humidity.

±0.1° C during all experiments while the humidity was varied. In the three series of experiments the latter was kept at 55±2.5, 67.5±2.5, and very close to 100 per cent. The number of birds in the corresponding groups was 7, 30, and 8. The first measurement was performed 10—15 seconds after the nestling had left the shell (cf. Tab. I). The means of the three groups are 39.36, 39.37, and 40.13° C. As the greatest difference between these means is not statistically significant we have treated the figures of all the 45 measurements as a homogeneous material with the mean 39.5±0.103° C as the body temperature at hatching.

The first condition for a raised metabolic activity in connection with the hatching is an increased supply of oxygen. As is well known the early irregular ventilation of the lungs is intensified when the young has made the first hole in the shell. According to our observations another 12—24 hours pass until the young proceeds to break the shell entirely with the bill (egg tooth?). Thereby it cuts the shell along a circle (Fig. 3 a) and with repeated efforts succeeds in prizing apart the two shell halves. This last phase of the hatching varies in length, depending on the activeness and strength of the

young. From our observations it is obvious that very slow and very rapid hatchings correspond to low and high body temperatures, respectively.

The observations of EYCLESHYMER (1907) and others suggest, that the heat production of the growing embryo increases slowly during incubation. The comparatively high temperature recorded by us at hatching must in addition be caused by the intense muscular activity in connection with the breaking of the shell and the low losses of heat as long as the young is enclosed in it. This conclusion is further supported by the findings (PREMBREY, GORDON & WARREN, 1894) that the CO_2-production increases during hatching.

Subsequently the body temperature of the nestlings was also taken 3, 7, 11, 15, 20, 25 and 35 minutes after hatching (cf. Tab. I). Fig. 2 illustrates the variation of the mean value of the body temperature in the three experiments with different humidity.

TABLE 1. Body temperature at different times after hatching in different relative humidity.

Air temp. °C	Rel. Humidity per cent	MINUTES AFTER HATCHING							
		0	3	7	11	15	20	25	35
37.3	55±2.5	38.7	36.1	34.1	33.4	33.2	33.2	33.2	34.0
		39.1	36.9	34.9	33.3	32.8	32.7	32.9	33.5
		38.7	35.8	34.9	33.3	33.0	32.9	33.3	33.8
		39.9	37.1	35.3	34.4	33.6	33.7	33.8	34.0
		40.0	36.8	35.3	34.2	33.6	33.2	33.6	34.1
		39.1	36.5	34.4	33.9	33.1	32.8	33.0	33.6
		40.0	37.0	35.1	34.3	33.5	33.1	33.2	33.9
MEANS		39.36	35.60	34.86	33.83	33.26	33.09	33.29	33.84
37.3	67.5±2.5	40.1	38.5	36.9	35.8	35.4	35.0	35.3	35.5
		40.5	38.3	36.4	34.9	34.6	34.3	34.4	34.6
		39.8	38.4	36.5	35.8	35.4	35.0	35.1	35.2
		40.5	38.4	36.6	35.6	35.2	34.9	35.1	35.3
		39.3	37.3	35.8	35.1	34.6	34.3	34.7	35.3
		40.0	37.8	35.4	34.2	33.5	32.9	32.9	33.0
		39.0[1]	37.6	37.1	35.5	35.4	35.5	35.2	35.6
		39.6	37.5	35.9	34.9	34.5	34.2	34.1	34.6
		39.5	36.8	35.5	34.4	33.6	33.5	33.4	33.9
		40.2[1]	37.5	35.7	36.3	35.5	34.9	35.3	35.3
		39.1	37.6	36.2	35.4	35.2	35.1	35.2	35.8
		38.5[1]	38.0	36.7	35.6	35.2	35.4	34.7	35.0
		38.8[1]	36.4	35.7	35.1	35.4	34.4	35.1	35.4
		39.5[1]	38.0	37.1	36.7	36.1	35.3	35.1	35.2
		38.5[1]	37.1	36.7	35.5	35.9	34.7	35.8	35.9
		38.4	36.6	34.6	33.5	33.2	33.1	32.8	33.5
		40.3	38.6	36.5	35.7	35.1	34.8	34.8	35.2
		39.4[1]	37.3	36.2	35.1	34.9	35.1	34.9	34.9

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		38.9	36.6	35.0	34.1	33.1	33.0	33.6	34.0
		39.2	37.5	35.4	34.9	33.7	33.5	33.6	33.9
		38.4	36.7	35.1	34.5	34.4	34.1	34.2	34.6
		39.9[1]	37.2	35.6	35.9	34.8	34.4	34.2	34.4
		38.7	37.1	35.5	34.6	34.2	34.1	34.1	34.3
		40.0	38.0	37.2	35.8	35.0	34.9	34.9	34.9
		39.2	36.3	35.1	34.5	34.0	33.8	33.7	34.5
		39.8[1]	37.4	36.3	35.3	35.9	34.7	34.6	35.3
		39.0	36.8	35.3	34.8	34.8	34.3	35.1	35.6
		39.0	36.5	35.6	34.8	34.4	34.1	33.9	34.3
		38.2	35.8	34.5	34.1	34.1	33.9	34.2	35.1
		39.8	36.3	35.3	34.4	34.2	34.1	34.2	34.7
MEANS		39.37	37.34	35.91	35.09	34.71	34.37	34.47	34.83
37.3	100	40.5	39.0	37.9	37.2	37.2	37.0	37.3	37.0
		40.8	39.1	38.1	37.1	37.4	37.0	37.0	37.2
		40.5	39.3	38.5	37.8	37.4	37.2	37.2	37.2
		40.4	39.4	38.3	37.9	37.8	37.4	37.1	37.1
		38.9	37.9	37.3	37.0	37.0	36.9	37.1	37.2
		40.0	38.9	38.2	37.9	37.7	37.1	37.1	37.2
		39.8	38.7	38.3	38.1	37.4	37.4	37.2	37.1
		40.1	39.1	38.4	37.9	37.3	37.1	37.0	37.0
MEANS		40.13	38.93	38.13	37.61	37.40	37.14	37.13	37.13

At heat balance, i.e. while the body temperature remains constant, the losses, chiefly due to radiation, conduction, convection, and evaporation, are equal to the gain of heat, the latter resulting from radiation, conduction, convection, and metabolic production. During periods of decreasing body temperature the losses dominate while at times of rising temperature the gain of heat preponderates over the losses. As seen from the curves in Fig. 2 the three experimental groups arrive at heat balance about 20 minutes after hatching by a drop in body temperature. In curve A evaporation is completely or very nearly eliminated. In consequence of this the drop is less steep than in the other curves and the body temperature is stabilized at a higher level. Compared with conditions before hatching, the nestling exposes a much greater surface to the air. Besides, this surface is covered with wet down (Fig. 3 a) with very poor insulating properties (cf. SMITH, 1939; LONG, 1948). In curves B and C, representing the body temperature in 67.5 and 55 per cent relative humidity, the drop occurs faster with lower relative humidity. This steeper fall must be due to heat losses by evaporation.

In curve A heat balance once established is maintained as long as the measurements are continued, i.e. during at least 15 minutes. This signifies that no important change occurs with regard to heat losses or heat gain. The temperature

[1] Individual with intense and irregular activity.

Fig 3 a. Newly hatched capercaillie nestling. b. Same nestling almost dry. Note appearance of the wet down in a and the drying down in b.

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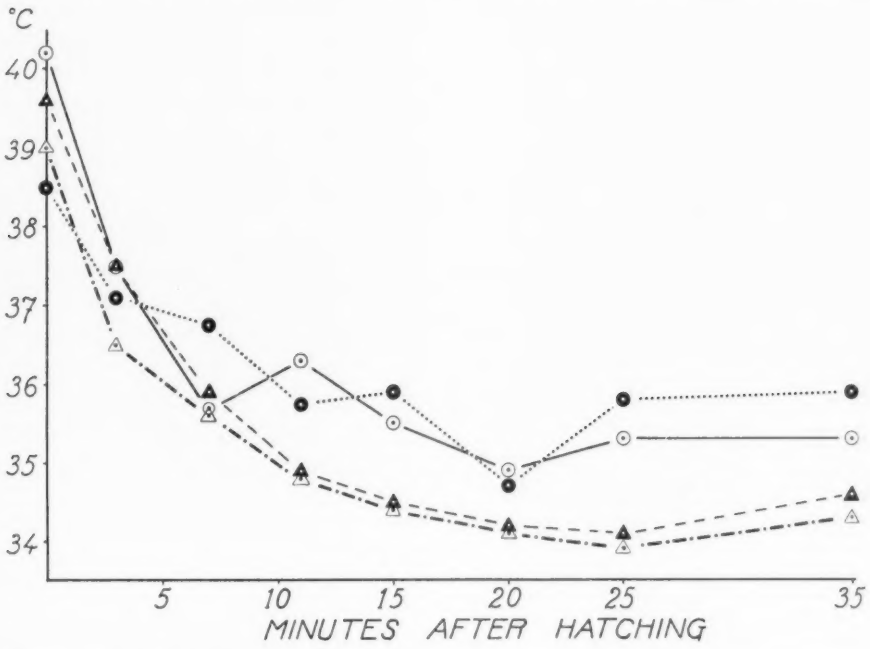

Fig. 4. Extreme individual temperature curves in 67.5 per cent humidity. Note difference between curves from calm (△) and from very active (⊙) chickens.

is stabilized about 0.2° C below that of the environment, this difference depending probably upon a small deficit in the saturation of the air with water vapour (cf. p. 44). In curve B and C heat balance is of very short duration, the temperature immediately beginning to rise. This implies either that the heat losses have diminished or that the heat gain has increased. A priori it seems most probable that the heat losses will become diminished because of the drying up of the down (Fig. 3 b) whereby the insulating properties of the latter will improve, and because of contraction of the blood vessels in the skin. To this comes possibly also a higher heat production from an increased muscular activity due to the cooling (for literature cf. LONG, 1948).

An evaluation of the relative importance of these factors during the rise in temperature is not possible. However, their potential rôle in the determination of the temperature level at heat balance in curve B and C may be estimated more easily. It must be stated that they all tend to stabilize the temperature at a higher level, and should be expected to come into action earlier and to a greater extent, the lower is the body temperature, i.e. earlier and more intensely in curve C than in curve B. Difficulties are encountered as soon as the metabolic heat production is concerned because of the considerable individual differences in the nestlings as to activity when crawling about and trying to rise. Changes

in activity, even during rather short periods, are, however, reflected in the individual temperature curves as shown in Fig. 4, although these irregularities disappear in the mean value curves (cf. Fig. 2).

If the temperature decrease in curve B and C below the corresponding value in curve A at heat balance (20 minutes after hatching) is due to evaporation it might be accounted for in the same way as is done in psychrometric measurements, using the simple theory that the psychrometric difference is proportional to the actual saturation deficit of the air sweeping past (cf. GRAMBERG, 1933). Expressing vapour pressures as relative humidities we obtain the formula

$$\frac{t - t_1}{1 - \Psi_1} = \frac{t - t_2}{1 - \Psi_2} = k$$

in which t, t_1 and t_2 designate body temperatures at relative humidities 1 (saturation), Ψ_1, and Ψ_2 respectively. For curve B we obtain k equal to 8.5, for curve C equal to 9.0. This is a fairly good agreement. However, the fact that a higher value of k is found at the lower relative humidity (cf. above) calls for a special inspection of the data upon which the value of k at the higher relative humidity is based. Here differences in activity between different individuals enter into discussion as this might involve a rather different heat production. In order to make a rough estimate in this direction the individual temperature curves are examined as to the occurrence of activity peaks. Unexpectedly we find individuals with such irregular curves only among those belonging to the group of curve B, the reason for this uneven distribution being unknown. This being the case, the actual temperatures of the three groups are not comparable. We have therefore excluded individuals with irregular temperature curves from the group of curve B and calculated mean temperatures for the 21 remaining ones. The curve obtained has its lowest point at 20 minutes after hatching with a mean temperature of 34.13° C. This value would correspond more closely to those for curve A and C. A calculation of k using this corrected value for curve B gives 9.3 which should be compared with that found for curve C (9.0).

This very good numerical accordance, the importance of which should not be over-estimated, suggests that the differences in temperature at heat balance between the three groups are chiefly due to evaporation. Unfortunately our data do not permit a statistical treatment. Further experiments are, however, in progress.

SUMMARY

The body temperature of nestlings of the capercaillie (Tetrao urogallus Lin.) has been measured 10—15 seconds after the nestlings leaving the shell at an

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environmental temperature of 37.3± 0.1° C. The mean of 45 measurements was 39.5± 0.103° C. This comparatively high temperature is referred to the muscular activity at hatching, the heat losses being very low as long as the young is enclosed in the shell. By studying the variation in body temperature during the 35 minutes after hatching at different relative humidities the decrease in temperature below that of the nestlings in 100 per cent was shown to be caused to a very great extent by the evaporation of water from the wet nestling.

ACKNOWLEDGEMENTS

The financial support of this investigation by the Swedish Game Research Council is gratefully acknowledged. We are very much indebted to the Swedish Hunting Commission for laboratory facilities. Our special thanks are due to Mr. Nils Höglund for directing our attention to the problem investigated, for his skilful co-operation, and for photographs of capercaillie nestlings put at our disposal. We thank Mr. H. Pettersson for valuable technical assistance.

LITERATURE

Eucleshymer, A. Ch. (1907). Some Observations and Experiments of the Natural and Artificial Incubation of the Egg of the Common Fowl. Biological Bulletin, Vol. XII, No. 6, 1907, pp. 360—375.

Gramberg, A. (1933). Technische Messungen. Berlin.

Höglund, N. H. (1955). Kroppstemperatur, aktivitet och föryngring hos tjädern *Tetrao u. urogallus* Lin. Viltrevy 1, pp. 1—87. Uppsala.

Long, W. H. Jr. (1948). The Physiology of Wild Gallinaceous Birds in Relation to the Environment. Thes. Degr. Ph. D. Univ. Michigan.

Prembrey, M. S., Gordon, M. H. and Warren, R. (1894). On the Response of the Chick, before and after Hatching, to the Changes of External Temperature. The Journal of Physiology, Vol. XVII, 1894—1895, No. 5, pp. 331—348.

Smith, R. J. (1939). A preliminary Study of the Body Temperature of the Ring-Necked Pheasant. Thes. Degr. Mast. Forest. Univ. Michigan.

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THE SYNCHRONOUS MITOTIC RHYTHM AND THE EFFECT OF LITHIUM DURING THE EARLY DEVELOPMENT OF THE SEA URCHIN EMBRYO

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It is generally accepted that during the earlier embryonic development a complete synchronicity occurs concerning the mitotic activity within the embryo and that this synchronicity subsequently disappears. Therefore, as a link in an investigation of the metabolism of the desoxypentose nucleic acid (DNA) during the embryonic development of the sea urchin embryo, the interest of the author was also directed to the changes in this mitotic activity. It seemed to be of importance to be able to state whether a relation possibly existed between the synchronous mitotic rhytm and the DNA-metabolism.

MATERIAL AND METHODS

For this investigation the sea urchin *Paracentrotus lividus* was used. For analyzing the mitotic rhythm a thorough analysis of the mitotic spectrum was made. From every series of developing embryos samples were taken out every fifth minute. A drop of the suspension was placed on a microscopic slide, a drop of aceto-orcein added and a coverglass placed without squeezing. By this technique the chromosomes stain very well, a three dimensional view of the embryo is obtained and thousands of embryos can be examined within a few hours. The preparation can be kept about a day before a complete overstaining occurs. In every sample the relative amount of embryos in inter-, pro-, meta-, ana- and telo-phase of the macro- and mesomeres was counted. The mitotic phases of the micromeres were not registered continously in the same manner, but these cells are to start with out of rhythm by about one phase. Prophase of the micromeres corresponds to metaphase of the macromeres, metaphase of the micromeres corresponds to ana-telophase of the macromeres and so on.

The normal mitotic spectrum was compared with the spectrum of Lithium-treated embryos. L iCl was used in a concentration of 0.565 M of which solution 10 parts were taken in 90 parts of sea water. The incubation of Li took place when the embryos had just entered the 2-cell stage.

The investigation was carried out at the Station Biologique Roscoff, France.

RESULTS

The changes in the mitotic spectrum are reproducted in figure 1. Each series of curves represents developing embryos from one single female. A number of shorter supplementary series were carried out in addition and all the series agree in all principal respects. The surface of each single curve represents a time sum and is an expression for the length of time the respective phase has taken.

From the curves it is obvious that the synchronous mitotic rhythm continues up to the 32-cell stage and that after this time the rhythm disappears between the single embryos of the same batch. After the 32-cell stage also the synchronicity within the single embryo is lost and cells in different mitotic phases are found at the same moment within one and the same embryo, which is easily seen by the microscopic examination of the embryos. This asynchronous mitotic activity within the embryo increases steadily after this time.

Also at the 32-cell stage another change in the mitotic spectrum can be seen. A general characteristic for the early embryonic cells during mitosis is their relatively long prophases in relation to metaphases. In the more adult cell the prophase has decreased in duration time and the metaphase increased. From the curves in figure 1 it is obvious that this change from a more embryonic state to a more adult state also occurs around the 32-cell stage.

Now it has been found that the embryos of the species used utilize stored cytoplasmic DNA for the formation of the nuclear DNA during the early embryonic development up to the 32-cell stage, AGRELL and PERSSON, at which time a sudden synthesis of DNA takes place in the embryo. Also the micromeres are formed just before this time at the entrance of the 16-cell stage. It may be asked whether it is the formation of the micromeres or the new synthesis of DNA or both, which changes the rhythm. By working with another species, *Psammechinus miliaris*, AGRELL (1955), it was found that in this species the new synthesis of DNA starts first at the 128-cell stage and the synchronicity continues also up to this stage, while the micromeres are as always formed at the 16-cell stage. Thus the formation of the micromeres is not related to the disappearing synchronous rhythm. This must depend upon the new synthesis of DNA.

In relation to these experiments it became interesting to see what effect Li may have upon the synchronicity in the embryos, as it is well known that Li upsets the regularity of the embryonic development by a process of "vegetalization". The results are given in figure 1. From the curves it is obvious that the effect of Li is a prolongation of of the synchronous rhythm, which now continues after the 32-cell stage up to around the 128-cell stage, after which time it progressively disappears both between the embryos in the same batch

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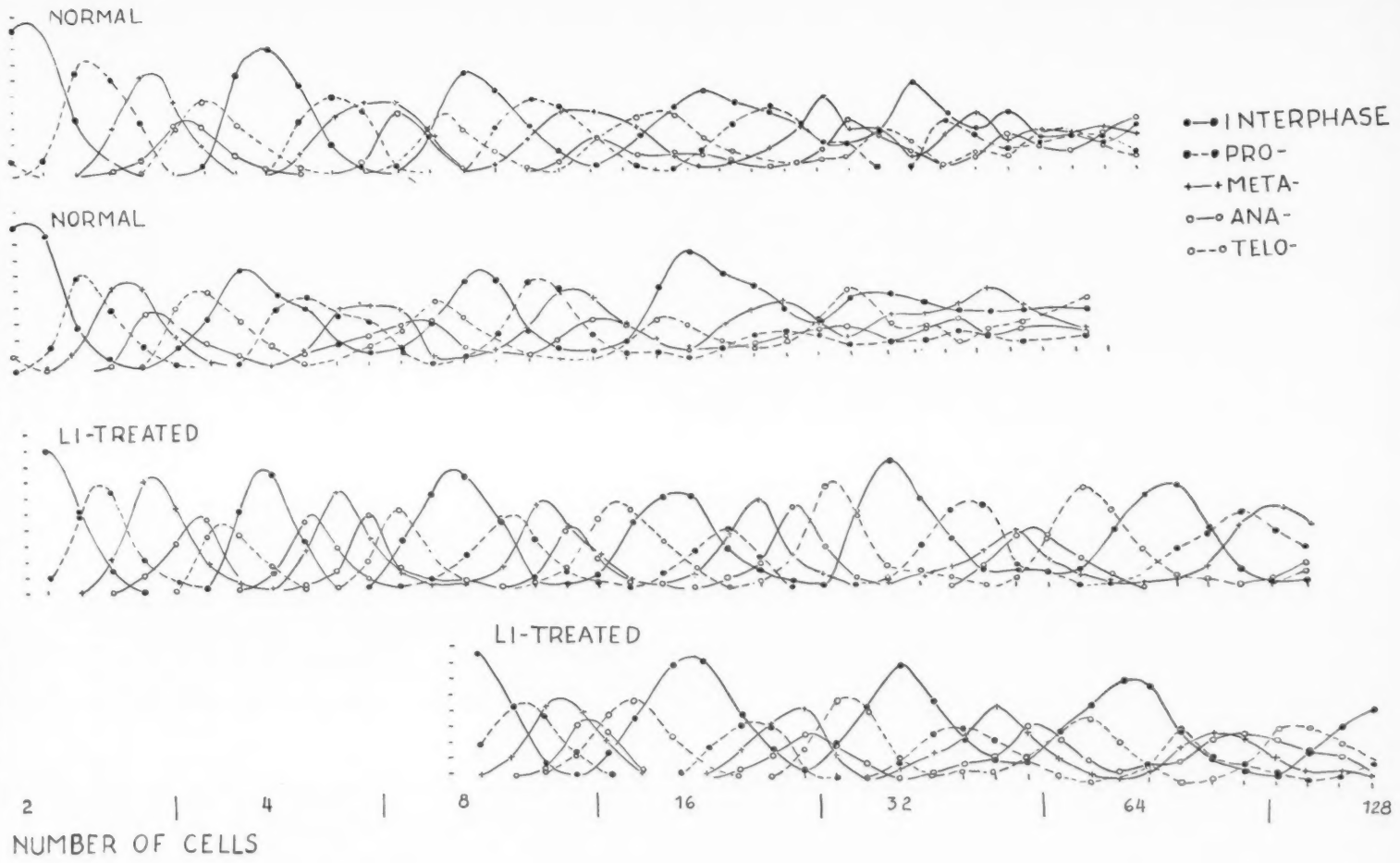

Figure 1. The mitotic spectrum of normal and Li-treated embryos. Horizontal axis: Time. Every interval 5 minutes. Vertical axis: Percental distribution. Every interval 10 per cent.

and within one and the same embryo. Also there is no change over from the embryonic to the adult type of the mitotic spectrum with respect to the relative length of prophase and metaphase after Li-treatment. However, the mitotic rate is unchanged as compared with the normal embryos.

The disappearence of the synchronous mitotic rhythm within a single embryo shows a certain interesting feature after Li-treatment. The cells in the different mitotic phases are not irregularily spread over the embryo but show a very distinct gradient reaching from the vegetal to the animal pole. Some photographs of such embryos are reproduced in plate 1. It can be observed that the cells at the vegetal pole always represent a later mitotic stage than the cells nearer the animal pole. In some embryos it is possible to find a whole gradient reaching from telophases at the base of the vegetal pole, around the micromeres, over anaphases and metaphases to prophases at the very tip of the animal pole. This phenomenon seems not to be restricted exclusively to Li-treated embryos. Also in normal embryos observed by the mentioned aceto-orcein method there is a certain tendency for the same gradient, but the definitive proof will represent a tedious statistical work. Also in embryos, i.g. blastulae, already asynchronous for a long time it would seem, that there is a high probability of finding several neighbouring cells in mitosis or even in the same mitotic phase, which might indicate a mutual interdependence of cells for their mitotic state.

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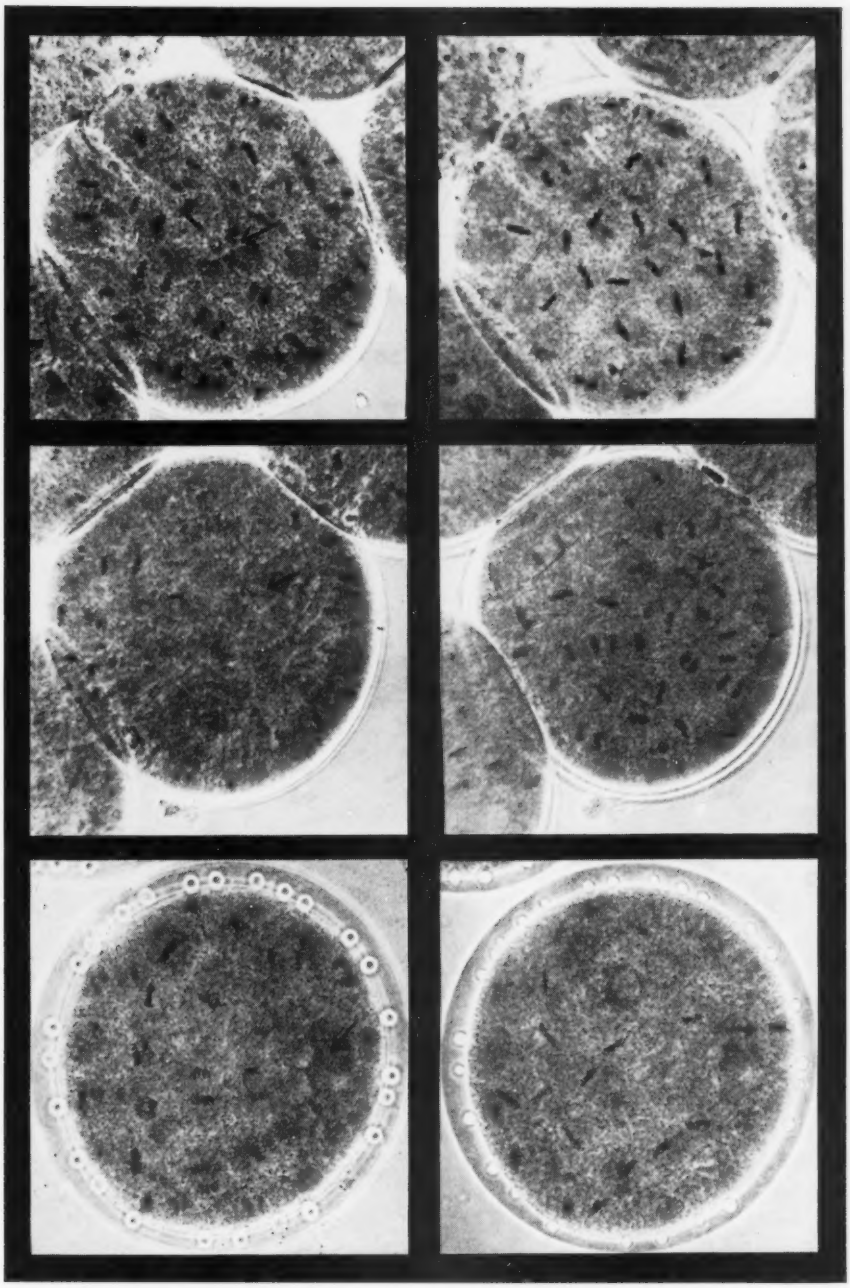
DISCUSSION

It has thus been shown that the synchronous mitotic rhythm characterizes the early embryonic development during the time when the embryos live upon their stored cytoplasmic DNA, but disappears at the point when the embryos commence a new synthesis of DNA, whether this starts at the 32-cell stage as in *Paracentrotus* or at the 128-cell stage as in *Psammechinus*. The reason is easy to understand. As long as DNA exists ready made and in excess in the form of cytoplasmic DNA the mitotic rate can run at full speed. The embryos are maximally embryonalized. At the same moment as the requirement of nuclear DNA starts depending on a new synthesis of DNA the establishment of this mechanism will represent a critical point in the development. Every embryo and also every cell will have to rely on its own capacity for this synthesis. The cells and the embryos start to individualize, to age. Differentiation can begin. This antagonism between mitosis and differentiation can also be seen, anew, later during the development, when with the formation of the mesenchyme blastula a mitotic gradient appears in the embryo from the vegetal to the animal pole, AGRELL (1954), which is reversed with respect to a mitochondrial gradient, GUSTAFSON and LENIQUE (1952).

VEGETAL POLE ANIMAL POLE



A

B

C

Plate 1. Li-treated embryos at the 64-cell stage with a gradient of the mitotic phases developed from the vegetal to the animal pole. The site of the micromeres is indicated by an arrow. Aceto-orcein preparation. Embryo A: Anaphases at the vegetal pole and metaphases at the animal pole. Embryo B: Telophases around the micromeres, followed by anaphases and metaphases at the animal pole. Embryo C: Late anaphases around the micromeres followed by early anaphases at the vegetal pole. Metaphases and at the tip of the animal pole prophases.

At the same time when the synchronous rhythm fades out, the mitotic spectrum of the embryo changes over from the embryonic type with relatively long prophase to the adult type with relatively long metaphases. Whether this may denote, that an incorporation of DNA in the chromosomes occurs at different mitotic phases during the two periods remains an open question.

The action of lithium was demonstrated to be a synchronizing effect upon the mitotic activity. This prolonged synchronicity which represents a continuation of the first embryonic period, continues for about two mitotic cycles longer than in normal embryos and must represent a prolonged embryonalization. As Li has an activating effect upon the mitosis in the early embryo the process of differentiation must be correspondingly delayed. The formation of ecto- and endoderm is a result of a differentiation. Thus it seems as if the overdevelopment of the endodermal part of the embryo and the underdevelopment of the ectodermal part, which appears later on after a Li-treatment, is primarily caused not by a process of vegetalization but by a process of embryonalization, during which the differentiation gradient in the embryo is smoothed out. That Li has a dedifferentiating effect can also be seen from the fact that Li-treated larvae show a much slower increase in the amount of mitochondria than normally developing larvae, GUSTAFSON and LENIQUE (1952). The formation of mitochondria must be regarded as a phenomenon following differentiation.

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As mentioned the mitotic synchronicity characterizes a period of utilization of cytoplasmic DNA and the non synchronous period starts when the cells commence to make a new synthesis. In accordance to these facts it was also found, by direct measurements of DNA during the development of Li-treated embryos, that the biochemical effect of Li represents a delay of the sudden new synthesis of DNA at the 32-cell stage and a prolonged utilization of cytoplasmic DNA, AGRELL and PERSSON.

When the period of synchronicity in the Li-treated embryos starts to disappear a gradient concerning the mitotic phases appears spreading from the vegetal to the animal pole. The cells at the vegetal pole represent always a later mitotic stage than the cells at the animal pole. This phenomenon, which can be traced also in normal embryos, must denote that the cells at the vegetal pole have started their mitosis earlier or pass the cycle faster than the cells at the animal pole. There seems to be no other possible explanation than that of assuming that the micromeres exert a certain activating effect upon the mitosis of the surrounding macromeres. These in their turn activate new mitosis in new cells and thus a mitotic wave spreads over the embryo from the vegetal to the animal pole. Thus, the observed gradient speaks definitily in favor of the existence of one gradient and against the view of the existence of a double gradient.

The fact that the micromeres have an influence upon the regulatory processes in the embryo is well known from the investigations of HÖRSTADIUS (1935). We

may consider now, that this influence represents an action upon the mitotic activity of the other cells. One may ask how the micromeres may exert their influence upon the mitotic activity of the macromeres. One of the few things we really know about the micromeres, which can be utilized in this connection, is that the micromeres are formed through an unequal cleavage and are thus much smaller, about 30 times in volume, than the macromeres. They thus contain a much smaller amount of cytoplasm for the same nucleus. This should denote that the micromeres have a much smaller storage of cytoplasmic DNA than the macromeres and are therefore forced to start a synthesis of nuclear DNA earlier than the macromeres. If so, it is easy to understand that the micromeres have an influence upon the mitotic activity of the macromeres, especially if these through the prolonged synchronicity by the action of Li, have a depleted storage of cytoplasmic DNA at the entrance of the critical period for the synthesis of the nuclear DNA. The macromeres are thus rendered more sensitive to the action of the micromeres, to metabolic products realeased from the DNA-metabolism in the micromeres.

To sum up, the differentiation can be demonstraded to start very early during the embryonic development, already during the so called period of cleavage. The driving forces for this differentiation seem to be an interplay between mitotic activities and the metabolism of cytoplasmic and nuclear DNA. It may be advantageous to approach other processes of embryonic differentiation with this aspect in mind.

ACKNOWLEDGEMENTS

The investigation was aided by grants from the Scandinavian Insulin Foundation, the Swedish Cancer Research Foundation and the Swedish Research Council for Natural Science. The author gratfully acknowledges these grants.

SUMMARY

The mitotic spectrum was studied during the early period of the embryonic development of the sea urchin *Paracentrotus lividus* in normal and in lithium-treated embryos.

It was found that the synchronicity of the mitotic phases disappears suddenly in normal embryos of this species at the 32-cell stage. This occurs at exactly the same time as the embryos change over from utilizing cytoplasmic DNA to a new synthesis of nuclear DNA.

Lithium was found to have a synchronizing effect upon the embryos. The synchronicity of the mitotic rhythm started to decline only after the 128-cell

stage. Thus the Li-effect represents a delay of differentiation by a prolonged mitotic activity. The biochemical effect of Li is a delay of the new synthesis of nuclear DNA and a prolonged utilization of the cytoplasmic DNA.

It was also found that after Li-treatment the micromeres exert a direct influence upon the mitotic activity of the macromeres when the asynchronicity develops. A mitotic gradient reaching from the vegetal to the animal pole appear.

LITERATURE CITED

AGRELL, I. 1954. A mitotic gradient in the sea-urchin embryo during gastrulation, Ark. Zool. *13*, 213.

— 1955. L'action du testosterone sur les premières phases du développement embryonnaire de l'oursin Psammechinus miliaris, C. R. Soc. Biol. *149*, 1322.

AGRELL, I. and H. PERSSON, Unpublished data.

GUSTAFSSON, T. and P. LENIQUE, 1952. Studies on mitochondria in the developing sea urchin egg, Exptl. Cell. Res. *3*, 251.

HÖRSTADIUS, S. 1900. Uber die Determination im Verlaufe der Eiachse bei Seeigeln, Pubbl. Staz. Zool. Napol. *14*. 251.

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BEITRÄGE ZUR ENTWICKLUNGSGESCHICHTE DES JACOBSONSCHEN ORGANS BEI *EMYS EUROPAEA**

VON

JOHANN LOEW

Mit 17 Abbildungen im Text.

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I. EINLEITUNG UND FRAGESTELLUNG; MATERIAL UND METHODE

Das Geruchsorgan und das JACOBSONsche Organ der Schildkröten hat SEYDEL (1896) erstmalig auf vergleichend-anatomischer Grundlage ausführlich bei *Testudo, Emys* und *Chrysemys* beschrieben. Nach seinen Befunden sollen drei Sinnesepithelbezirke im Bereich des respiratorischen Epithels der Nasenhöhle dem JACOBSONschen Organ der übrigen Tetrapoden entsprechen. MC COTTER (1917) hat bei *Chrysemys* sogar vier Sinnesepithelabschnitte in der pars respiratoria beschrieben, die er als einzelne Abschnitte eines JACOBSONschen Organs deutet.

Seither sind trotz mehrfach in der Literatur aufscheinender Kritiken und Zweifel, die vor allem MIHALKOVICS (1899) und FUCHS (1915) geltend machten, keine weiteren, die offenen Probleme klärenden Untersuchungen angestellt worden. Andererseits sind die SEYDELschen Befunde als maßgebend in der späteren Literatur (PETER 1899; MATTHES 1936) berücksichtigt worden. Es erschien daher eine neue Untersuchung angebracht.

Das Problem der Entwicklung des JACOBSONschen Organs steht im Vorder-

*) Die Arbeit wurde in den Jahren 1950—53 im Zoologischen Institut der Wiener Universität ausgeführt. Dem inzwischen verstorbenen Vorstand des Institutes, Herrn Prof. STORCH, sowie dem derzeitigen Leiter, Herrn Prof. MARINELLI danke ich für die Überlassung eines Arbeitsplatzes und die Förderung der Arbeit. Herrn Doz. Dr. H. HOFER bin ich für die Anregung zu dieser Arbeit, für die Überlassung des Materials, welches er aus der Sammlung von Herrn Prof. HOCHSTETTER erhielt, und für die Unterstützung durch wertvolle Ratschläge zu Dank verpflichtet. Herrn Dr. ADAM danke ich für einige Hinweise, die Anfertigung der Mikrophotographien und die Überarbeitung des Manuskriptes.

grund der folgenden Ausführungen. Doch wurden auch andere, bisher nicht erwähnte Vorgänge in der Entwicklungsgeschichte des Geruchsorgans von *Emys europaca* beachtet, welche die Muschelbildung und die Genese des Tränennasenganges betreffen. Bei der Beschreibung des Befunde wurde die von FUCHS vorgeschlagene Terminologie verwendet. Zur genaueren Kennzeichnung der einzelnen Entwicklungsstufen wurde bei jedem Embryo auch der Zustand der Augenentwicklung angegeben.

Untersucht wurden Sagittal- und Querschnittserien von 10 Köpfen verschieden alter Embryonen der europäischen Sumpfschildkröte *(Emys europaca)*. Für Vergleichszwecke wurden einige Querschnittserien von Ringelnatter-Embryonen *(Natrix natrix)* angefertigt. Die Schildkröten waren in BOUINscher und die *Natrix*-Embryonen in ZENKERscher Flüssigkeit fixiert. Die Kopflänge wurde unter der Lupe auf Millimeterpapier vermessen. Die starke Krümmung der jüngsten Embryonen erschwert die Feststellung der tatsächlichen Kopflänge; wir geben daher hier als "Kopflänge" den Abstand zwischen Schauzenspitze und Mittelhirnhöcker wieder. Die grösseren Köpfe wurden in 5 %iger wässriger Salpetersäure entkalkt. Einbettung erfolgte in Celloidin-Paraffin. Die Schnittdicke betrug bei den jüngeren Embryonen 6—8 μ, bei den älteren mit bereits ausgebildeten Knorpel- und Knochenelementen 8—16 μ. Die Präparate wurden mit Hämalaun-Eosin, Azan (HEIDENHAIN), Eisenhämatoxylin und MALLORY gefärbt.

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II. BESCHREIBUNG DER BEFUNDE

Embryo von Emys europaca mit Kopflänge 1,5 mm. (Abb. 1).*

Bei diesem jüngsten von uns untersuchten Embryo sind die beiden Riechplakoden bereits zum Riechgrübchen eingesenkt. Dieses hat die Gestalt einer annähernd parallel zur Sagittalen an der Lateralseite des Vorderkopfes verlaufenden Rinne, deren Tiefe occipitalwärts allmählich abnimmt. Es wird vom äusseren (lateralen) und inneren (medialen) Nasenfortsatz begrenzt und besitzt eine Länge von ungefähr 120 μ. Der äussere Nasenfortsatz schliesst occipitalwärts einen zunehmend stumpfer werdenden Winkel mit der Medianebene ein und verliert allmählich an Masse. Nach rostral zu bildet das Riechgrübchen eine seichte Bucht in das Mesoderm des Vorderkopfes. (Abb. 1 a, 2). Bis auf diese Eindellung stimmt dieses Stadium mit dem jüngsten von SEYDEL beschriebenen Embryo I von Chrysemys picta mit 4,5 mm Scheitel-Steißlänge überein. Auf seiner Abbildung liegt jedoch diese Eindellung bereits an einer tieferen Stelle des Riechgrübchens. Die mehr rostral gelegenen Schnitte wurden von ihm augenscheinlich nicht beachtet.

[1] Alle Abbildungen wurden gegenüber den Originalen auf ⅔ verkleinert.

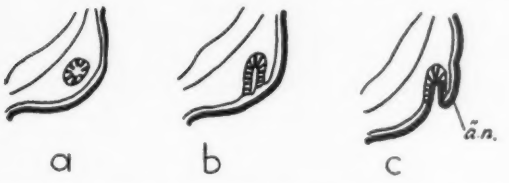

Abb. 1. Embryo von *Emys europaea*. *Kopflänge* 1,5 mm. Querschnitte durch das Reichgrübchen einer Körperseite. ä.n. äußerer Nasenfortsatz. 48fach.

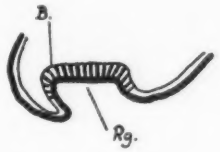

Abb. 2. *Emys europaea*. Kopflänge 1,5 mm. Sagittalschnitt durch die Nasenmundrinne. B. rostrale Bucht; Rg. Riechgrübchen. 48fach.

Ein Sagittalschnitt durch die Nasenmundrinne eines Embryos gleicher Grösse zeigt ebenfalls deutlich die beschriebene, rostral gerichtete Bucht gegen das Mesoderm (Abb. 2). Das Riechgrübchen ist also in der Tiefe länger als die äusserlich sichtbare Nasenmundrinne.

Das Riechgrübchen wird vollständig von gleichartigem und verhältnismäßig niedrigem embryonalen Riechepithel ausgekleidet, welches an den ventralen Rädern der Nasenfortsätze ohne deutliche Grenze in das benachbarte Ektoderm übergeht. Die Riechgrübchen sind durch das sehr breite primäre Nasenseptum voneinander getrennt.

Das Auge, dessen Entwicklungsgrad wir als Vergleich immer anführen werden, ist als primärer Augenbecher ausgebildet. Das eben vom Ektoderm abgeschnürte, hohle Linsenbläschen hat Bohnenform. Die Retina liegt als hohes undifferenziertes Zylinderepithel vor.

Embryo von Emys europaea mit Kopflänge 2,0 mm. (Abb. 3).

Die vorliegende Querschnittserie zeigt wesentliche Änderungen gegenüber dem vorher beschriebenen Stadium. Das Sinnesepithel des Riechgrübchens ist bedeutend in die Tiefe verlagert und ist nicht mehr oral, sondern occipital am tiefsten. Diese Veränderungen sind auf das Vorwachsen und teilweise Verschmelzen des Oberkieferfortsatzes mit dem äusseren Nasenfortsatz zurückzuführen. Die Grenze zwischen den beiden Fortsätzen ist an weiter caudal gelegenen Schnitten (Abb. 3 b) zu erkennen und als Knickung im Ektoderm des Integuments und Riechgrübchens von entwicklungsgeschichtlich besonderer Bedeutung. Hier wird der Tränennasengang als Epithelleiste durch Abschnürung vom Ektoderm der Haut angelegt. Diese Epithelleiste sinkt später in die Tiefe des Mesoderms ein, wird rohrförmig und stellt die definitive Beziehung zwischen Auge und Nasenhöhle her (Born 1879).

Im caudalen Abschnitt legt sich der Oberkieferfortsatz an den als "Vomerpolster" (Fuchs) bezeichneten Abschnitt des späteren Daches der Mundhöhle an. Das Riechgrübchen besitzt eine Länge von ungefähr 180 μ. Die Beteiligung des Oberkieferfortsatzes an der Bildung der lateralen Wand des Riechgrüb-

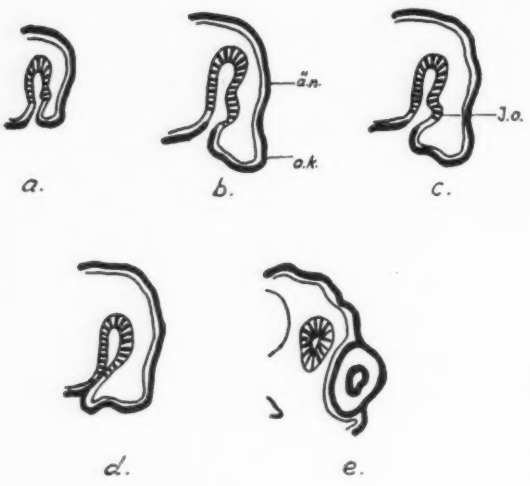

Abb. 3. *Emys europaea*. Kopflänge 2,0 mm. Querschnitte durch das Riechgrübchen mit JACOBSONschem Organ (J.o.); o.k. äußerer (lateraler) Oberkieferfortsatz. Schnittfolge a—e von oral nach caudal. 48fach.

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chens ist von wesentlicher Bedeutung für dessen epitheliale Bekleidung: es wird dadurch "indifferentes" Ektoderm in die epitheliale Auskleidung des Riechgrübchens mit einbezogen.

Die Bezeichnung "indifferentes Epithel" wird in der Literatur häufig gebraucht, jedoch nirgends eindeutig definiert. Zweifellos soll damit jedoch der Unterschied zwischen dem vom Ektoderm stammenden, aus primären Sinneszellen bestehenden Sinnesepithel und dem in den frühen Stadien noch undifferenzierten Ektoderm im Riechgrübchen betont werden. In der weiteren Entwicklung differenziert sich nämlich dieses "indifferente" Epithel zum respiratorischen Epithel der Nasenschleimhaut. Die Verteilung der beiden Epithelien an der lateralen Wand wird von der Gestalt des Oberkieferfortsatzes bestimmt. Dieser ist oralwärts, wo er sich mit dem Stirnfortsatz vereinigt am geringsten entwickelt, occipital am stärksten. An den vordersten Querschnitten (Abb. 3 a, b) ist daher die Beteiligung indifferenten Epithels an der Auskleidung des Riechgrübchens nur gering und nimmt occipitalwärts allmählich zu (Abb. 3 d, e).

Die spaltförmigen Räume des paarigen Riechgrübchens haben sich einander genähert und es ist dadurch eine Verschmälerung des primären Nasenseptums festzustellen. Ähnliche Verhältnisse hat COHN (1903) bei Gallus als "Wandern des Riechgrübchens von der Lateralseite auf die Ventralseite des Vorderkopfes" gedeutet. Da jedoch bei Veränderungen der Lage und Gestalt einzelner Teile während der Entwicklung nicht nur ein Abschnitt aktiv ist, sondern vielmehr eine gegenseitige Beeinflussung der einzelnen Abschnitte erfolgt, dürfte die Lageveränderung des Riechgrübchens nicht auf eine einzige und ausschließliche Ursache zurückzuführen sein.

Das Auge ist grösser geworden und zeigt deutliche Entwicklungsfortschritte. Die Linse besitzt nur mehr einen schmalen spaltförmigen Hohlraum und ist vom Ektoderm durch Mesodermzellen getrennt.

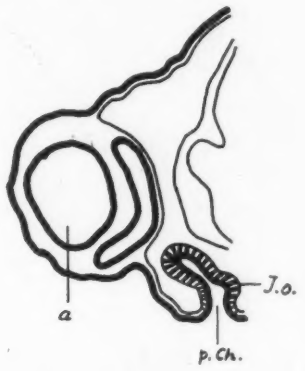

Abb. 4. *Emys europaea.* Kopflänge 2,5 mm. Ausgewählter Querschnitt durch Riechgrübchen und Auge (a); J.o. JACOBSONsches Organ. p.Ch. primitive Choane. 48fach.

Embryo von E m y s e u r o p a e a mit 2,5 mm Kopflänge. (Abb. 4).

Die Grenze zwischen dem lateralen Nasenfortsatz und dem Oberkieferfortsatz ist bei diesem Embryo bereits undeutlich, da die beiden Fortsätze mehr und mehr zu einer einheitlichen Masse verschmelzen. Ausserdem sieht man auf den Querschnitten, daß das Lumen des Riechgrübchens stark nach lateral geneigt ist. Möglicherweise hat auf diese Neigung das überaus starke Wachstum des Auges Einfluß; es nimmt in diesem Stadium fast den ganzen Bereich des Vorderkopfes ein. An der mit Sinnesepithel ausgekleideten Wand ist eine ungefähr 80 μ lange, medial gerichtete schwache Eindellung feststellbar.

Für die Beurteilung aller weiteren Entwicklungsvorgänge der Nasenanlage war es nun sehr wichtig, die Frage zu klären, ob es sich bei dieser medialen Eindellung um die Anlage des JACOBSONschen Organs handelt. Es wird für alle bisher untersuchten Sauropsiden als Eindellung der medialen Wand des Riechgrübchens angelegt. Um unsere Annahme, daß es sich bei der medialen Eindellung des Riechgrübchens von *Emys europaea* und die Anlage des JACOBSONschen Organs handelt zu festigen, wurden einige Embryonen von *Natrix natrix* eines ähnlichen Entwicklungsstadiums untersucht. Die Entwicklungsgeschichte des JACOBSONschen Organs bei den Squamaten kann als durchaus geklärt gelten und ist von den ersten Anlagen bis zur Ausbildung bei der adulten Form gut bekannt. Die adulten Squamaten besitzen ein von der Haupthöhle völlig getrenntes, mit eigenem Hohlraum und Ausführungsgang versehenes JACOBSONsches Organ, welches durch Abschnürung von der medialen Wand gebildet wird. Eine Querschnittserie durch den Kopf eines *Natrix*-Embryos von 3 mm Kopflänge läßt erkennen, daß auch hier die Anlage des JACOBSONschen Organs als Rinne vorliegt. Der Oberkieferfortsatz ist jedoch noch nicht am Aufbau der lateralen Wand beteiligt. (Abb. 5). Ein etwas älterer *Natrix*-Embryo von 4 mm Kopflänge zeigt die rasch fortschreitende Abschnürung des JACOBSONschen Organs von der Haupthöhle (Abb. 6). Hier sieht man bereits die Beteiligung des sehr grossen Oberkieferfortsatzes am Aufbau der lateralen Wand des Riechgrübchens.

Vergleicht man die Anlage des JACOBSONschen Organ des beschriebenen Embryos von *Emys* mit 2,5 mm Kopflänge mit dem von *Natrix* mit 3 mm Kopflänge, so ergibt sich, daß die Anlage des Organs offenbar bei den Schildkröten relativ später erfolgt. Schon PETER (1899) hat auf diesen Umstand hingewiesen, bezweifelt jedoch, ob es sich bei dieser medianen Eindellung von Emys tatsächlich um die Anlage des JACOBSONschen Organs

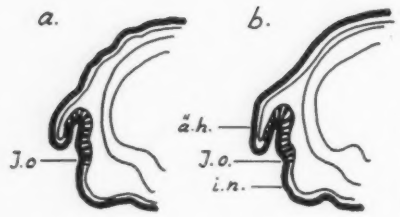

Abb. 5. Embryo von *Natrix natrix*. Kopflänge 3,0 mm. Anlage des JACOBSONschen Organs (J.o.) in der medialen Wand des Riechgrübchens. i.n. innerer (medialer) Nasenfortsatz; ä.n. äußerer Nasenfortsatz. 48fach.

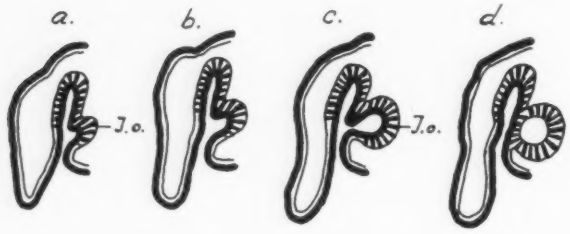

Abb. 6. *Natrix natrix*. 4 mm Kopflänge. Abschnürung des JACOBSONschen Organs (J.o.) von der Haupthöhle. 48fach.

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handelt. Diese Kritik PETERS stützt sich jedoch nicht auf eigene Untersuchungen, sondern auf die Arbeit von SEYDEL.

Wir glauben jedoch, daß auf Grund unserer eigenen Untersuchungen nun klar hervorgeht, daß diese Anlage tatsächlich einem JACOBSONschen Organ entspricht; es wird also bereits vor der Choanenbildung angelegt. Diese Annahme wird durch eine bisher nicht erwähnte und beachtete Tatsache bestärkt: nicht nur das JACOBSONsche Organ, sondern auch die Muschel und der Tränennasengang als typische Bauelemente der Nasenhöhle werden bei *Emys* ontogenetisch relativ später als bei den Squamaten angelegt.

Ein Vergleich des JACOBSONschen Organs von *Emys* europaea mit entsprechenden Entwicklungsstadien der Vögel konnte nur auf Grund von Angaben in der Literatur durchgeführt werden. Der erste kurze Bericht über ein JACOBSONsches Organ bei Vögeln stammt von GANIN (1890). COHN (1903) beschreibt bei einem *Gallus*-Embryo von 5,6 mm Kopflänge ein Rudiment eines JACOBSONsches Organs, welches in der späteren Entwicklung vollständig rückgebildet wird. BEECKER (1903) will einer "so vergänglichen Eindellung" keinen morphologischen Wert zusprechen. Die diesbezüglichen Verhältnisse bei den Vögeln bedürfen jedoch offenbar einer erneuten Untersuchung. Erst kürzlich konnte GREWE (1951) bei *Anas sp.* eine Anlage des JACOBSONschen Organs, die nur vorübergehend in der Ontogenie auftritt, feststellen.

Der ontogenetische Vergleich des JACOBSONschen Organs von Squamaten *(Natrix natrix)* und Testudinaten *(Emys europaea)* ergibt, daß dieses Organ bei letzteren als rinnenförmige Einsenkung in der medialen Wand der Nasenmundrinne auftritt und in Lage und Gestalt grundsätzlich mit den Verhältnissen bei anderen Amnioten übereinstimmt. SEYDEL nimmt auf Grund des Vergleichens mit den Amphibien eine viel umfangreichere Anlage des JACOBSONschen Organs bei Emys an. Wir glauben jedoch, daß dieser Ver-

gleich von SEYDEL unrichtig ist, da zunächst die Verhältnisse in phylogenetisch einander näher stehenden Gruppen, also zunächst innerhalb der Reptilien und dann der Amnioten untersucht und verglichen werden müssen.

Die epitheliale Auskleidung des Riechgrübchens entspricht den Verhältnissen bei dem vorher beschriebenen Stadium. Die mediale Wand ist vollständig von Sinnesepithel ausgekleidet, der ventrale Abschnitt der lateralen Wand von indifferentem Epithel.

Das Auge zeigt weitere Fortschritte der Differenzierung. Das Pigmentepithel liegt der Retina unmittelbar an, die Pigmenteinlagerung ist schon deutlich im Gange. Die Linse besitzt keinen Hohlraum mehr und die Ränder der Augenblase beginnen über sie hinwegzuwachsen.

Bei der Besprechung der folgenden Stadien werde ich, soweit es für das Verständis der sich ändernden Lagebeziehungen des JACOBSONschen Organs erforderlich erscheint, auf die Bildungsweise des Gaumens eingehen. Eine ausführliche Auseinandersetzung mit dem Problem der Gaumenbildung findet sich bei FUCHS (1907; 1908 und 1916).

Embryo von E m y s e u r o p a e a von 2,5 mm Kopflänge. Sagittalschnittserie.

Die Abb. 7 läßt deutlich die Bildung des primitiven Gaumens erkennen. Der Schnitt 7 a geht durch den nach lateral geneigten Abschnitt der Nasenmundrinne (vergl. Querschnitte, Abb. 4) auf Schnitt 7 b ist die äussere Nasenöffnung und auf Schnitt 7 c die primitive Choane getroffen. Es handelt sich hier natürlich nur um einzelne, durch zahlreiche dazwischenliegende Schnitte getrennte, ausgewählte Abschnitte durch die Organanlage. Auf Abb. 7 c ist zwischen der äusseren Nasenöffnung und der primitiven Choane die in Bildung begriffene Gaumenbrücke sichtbar, die durch Verschmelzen des äusseren und inneren Nasenfortsatzes zustande kommt. Die Choane und die äussere Nasenöffnung sind noch durch einen schmalen Spalt miteinander verbunden, äusserer und innerer Nasenfortsatz stehen also kurz vor ihrer Vereinigung.

Dieser Embryo weist zwar die gleichen äusseren Maße wie der vorhin als Querschnitt besprochene Embryo auf, ist jedoch in seiner Entwicklung schon weiter fortgeschritten. Die aus der Literatur bekannte Tatsache, daß äussere Maße und tatsächliche Entwicklungsstufe oft nicht übereinstimmen, kann ich hier bestätigen.

Das JACOBSONsche Organ mündet als Rinne in seiner ganzen Ausdehnung in die Nasenmundrinne. Dieses Stadium stimmt bis auf geringfügige Unterschiede mit dem Embryo II von SEYDEL (p. 467) überein.

Embryo von E m y s e u r o p a e a mit 3,0 mm Kopflänge. (Abb. 8).

Auf einer Querschnittserie ist die Ausbildung des primitiven Gaumens nun in seinem ganzen Umfang zu erkennen. Die Nasenfortsätze liegen mit ihren

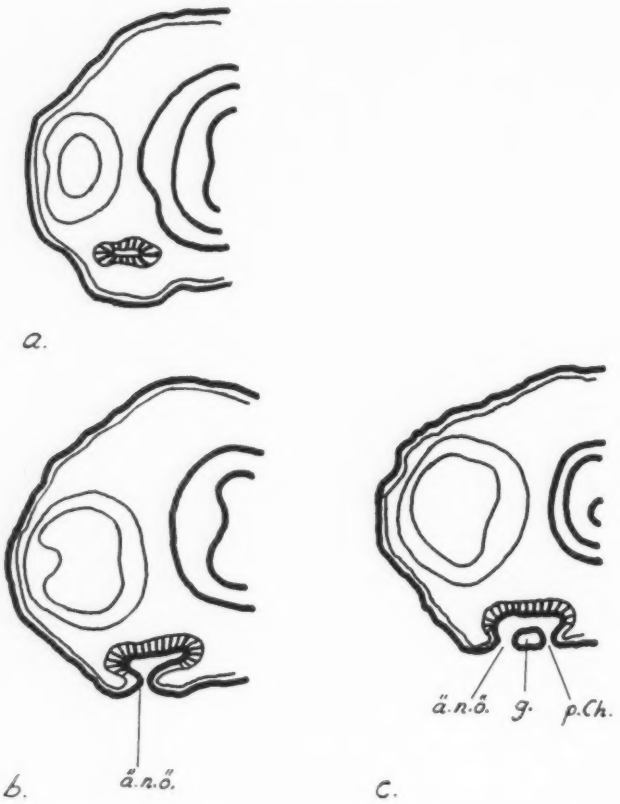

Abb. 7. *Emys europaea*. 2,5 mm Kopflänge. Ausgewählte Schnitte einer Sagittalschnittserie. ä.n.ö. äußere Nasenöffnung; g. Gaumen; p.Ch. primitive Choane. 48fach.

Epithelien unmittelbar aneinander, jedoch sieht man an keiner Stelle des Verklebungsbereiches eine Auflösung der Epithelien. Der primitive Gaumen wird jedoch nicht ausschliesslich als Verschmelzungsprodukt der äusseren Nasenfortsätze gebildet. Es zeigt sich, daß an seiner Bildung der Oberkieferfortsatz und die äusseren Nasenfortsätze als einheitliche Masse unter Einbeziehung von indifferentem Epithel beteiligt sind. Abb. 8 a zeigt die äussere Nasenöffnung, der weiter caudal liegende Schnitt der Abb. 8 e die primitive Choane. Das auf Abb. 8 b deutlich als Rinne erkennbare JACOBSONsche Organ mündet also nach der Bildung des primitiven Gaumens in die primitive Choane (Abb. h).

Dieses Stadium stimmt mit dem von FUCHS (1915, p. 208) beschriebenen Embryo von 3,6 mm Nacken-Steißlänge fast vollkommen überein. Man sieht dort ebenfalls eine Rinne in der medialen Wand, die caudalwärts zunehmend seichter wird. Sie wurde von FUCHS nicht näher beschrieben, ihre Identität mit der an dem von uns beschriebenen Embryo als JACOBSONsches Organ bezeich-

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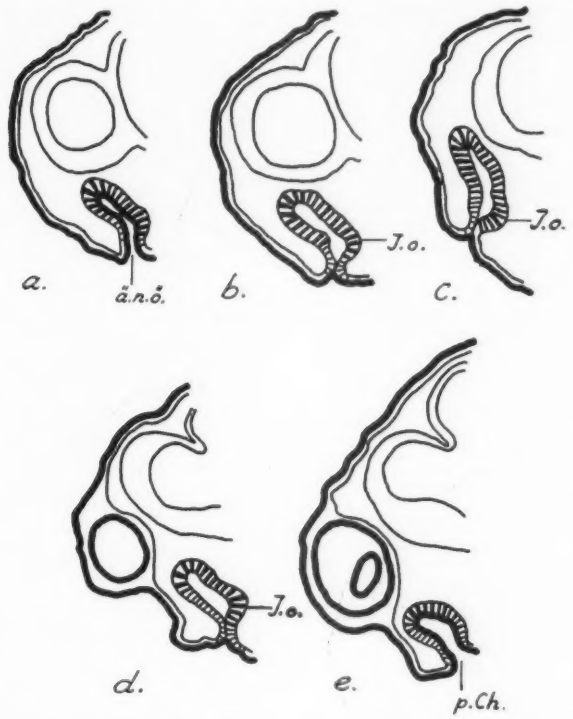

Abb. 8. *Emys europaea.* 3,0 mm Kopflänge. Ausgewählte Schnitte einer Querschnittserie; a—e von oral nach caudal. Schnitt a mit äußerer Nasenöffnung (ä.n.ö.). Schnitt f mit primitiver Choana (p.Ch.); JACOBSONsches Organ (J.o.). 48fach.

neten Eindellung kann jedoch nicht bezweifelt werden. Der von uns beschriebene Embryo ist, soweit aus den Abbildungen und Beschreibungen hervorgeht, auch mit dem von SEYDEL beschriebenen Embryo III *(Chrysemys picta)* vergleichbar.

Das Auge ist in diesem Stadium gegenüber den vorhergehenden deutlich vergrössert, zeigt jedoch sonst keine besonders auffallenden Veränderungen.

Bevor die weiteren Vorgänge der Gaumenbildung und die damit zusammenhängenden Umbildungen des Nasenschlauches besprochen werden, möchte ich auf einen für die Beurteilung der älteren Stadien wesentlichen Umstand hinweisen. Der Gestaltwandel der Nasenschleimhaut während der Entwicklung ist so bedeutend, daß es u. E. nicht genügt allein die wechselnden Reliefbildungen zu beschreiben und sie als mutmaßliche Abgliederungen des Sinnesepithelabschnittes des bisher beschriebenen JACOBSONschen Organs zu deuten; wir glauben, daß man den Schwierigkeiten, die sich einer Identifizierung des JACOBSONschen Organs entgegenstellen, nur dadurch begegnen kann, daß man schon in den frühesten Stadien die Beziehungen des Epithels zum Olfactorius berücksichtigt. SEYDEL behauptet, daß sich der Sinnesepithelbezirk des JACOBSONschen Organs auch auf den Boden und die laterale Wand der Nasenhöhle ausdehne. Die drei von ihm unterschiedenen Abschnitte des JACOBSONschen Organs seien durch schmale Streifen indifferenten Epithels getrennt. Eine

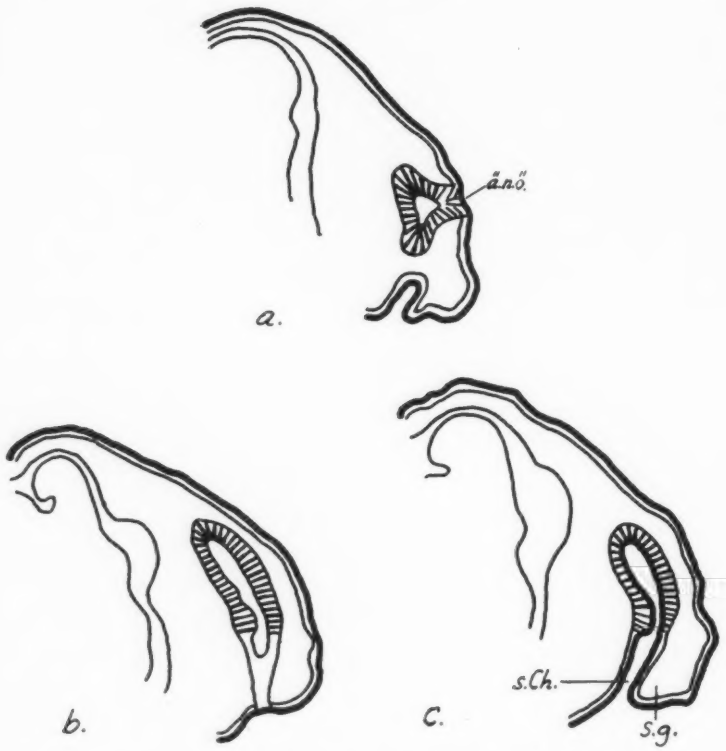

Abb. 9. *Emys europaea*. 3,5 mm Kopflänge. Ausgewählte Sagittalschnitte a—c von oral nach caudal. Bildung des sekundären Gaumens (s.g.) und der sekundären (defintiven) Choane (s.Ch.); ä.n.ö. äußere Nasenöffnung. 48fach.

solche Gliederung des JACOBSONschen Organs in drei Abschnitte würde einen völlig einmaligen und isolierten Typus innerhalb der Amnioten darstellen. Eine Untersuchung der Nervenversorgung mag hier u. E. einen genaueren Aufschluß zu geben.

Embryo von Emys europaea mit 3,5 mm Kopflänge. Sagittalschnittserie. (Abb. 9).

Diese Serie gibt über die fortschreitende Ausbildung des Gaumens, nunmehr bereits des sekundären, weitere Aufschlüsse. Der auf Abbildung 9 a wiedergegebene Schnitt zeigt die äussere Nasenöffnung, auf Schnitt 9 c ist der Nasengaumengang getroffen. Ein Vergleich mit dem auf Abb. 7 abgebildeten Embryo von 2,5 mm Kopflänge läßt die durchgreifenden Veränderungen im Aufbau des Munddaches erkennen, der im Wesentlichen auf der Beteiligung des Oberkieferfortsatzes an der Bildung des sekundären Gaumens beruht. Die in der Medianen verschmelzenden Oberkieferfortsätze verlängern das Lumen des Nasenschlauches durch den von ihnen gebildeten Nasengaumengang, der

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die primitive Choane weit nach caudal verschiebt. Erst in diesem Stadium kann daher von einer sekundären Choane, die sich dann bis zum adulten Tier erhält, gesprochen werden. Den grössten Anteil an der Gaumenbildung hat der Oberkieferfortsatz.

Die Bedeutung des sekundären Gaumens für die Lagebeziehungen und -veränderungen des JACOBSONschen Organ ist beachtlich; das Organ liegt nunmehr vollständig im Lumen der Nasenhöhle und hat seine Beziehungen zur primitiven Choane und damit zur Mundhöhle durch die Verlagerung der Choane in caudaler Richtung verloren. (Bezüglich der primitiven Choanen und die Lage der Mündung der JACOBSONschen Organe der Reptilien s.a. FUCHS 1907/1908).

Während bei den adulten Squamaten der Ausführungsgang des JACOBSONschen Organs noch als Rest der primitiven Choane erhalten ist, ist dies bei *Emys europaea* nicht der Fall. Der sekundäre Gaumen ist hier eine unmittelbare Fortsetzung des primitiven Gaumens ohne Aussparung eines Restes der primitiven Choane.

Embryo von Emys europaea mit Kopflänge von 3,5 mm. Querschnittserie. (Abb. 10).

Dieser Embryo weist gegenüber den früheren Stadien wieder einige bedeutende Veränderungen auf. Die vordersten Schnitte zeigen den Beginn der Ausbildung des Einführungsganges, dessen epitheliale Auskleidung von ektodermalen Epithelzellen übernommen wird, die etwas höher als die der Körperoberfläche sind. Im Bereich des Nasenschlauches können nur zwei verschiedene Reliefbildungen unterschieden werden, eine an der medialen Wand, das JACOBSONsche Organ, Abb. 10 a, b, c, und die andere weiter caudal an der lateralen Vorbuchtung gegen das Lumen der Nasenhöhle, die Muschelanlage. (Abb. 10 d). Das Problem der Nasenmuschel ist bei den Schildkröten noch nicht eindeutig geklärt. *Testudo* besitzt nach SEYDEL sicherlich eine echte Muschel, *Chelone* jedoch nur eine schwache Vorragung an der lateralen Wand, die von einer schmalen und dünnen Leiste der knorpeligen Nasenkapsel gestützt wird (FUCHS). Wenn die von BORN (1883) gegebene Definition der Muschel zurecht besteht, wonach die "Muschel als Vorragung der lateralen Wand sowohl ontogenetisch als auch phylogenetisch älter ist als der in ihr enthaltene Knochen oder Knorpel", dann liegt auch in dem vorliegenden Stadium von *Emys* eine echte Muschel vor. Sie tritt aber, ebenso wie das JACOBSONsche Organ, ontogenetisch relativ sehr spät auf; bei den Sauropsiden ist im allgemeinen die erste Anlage der Muschel bekanntlich bereits vor der Gaumenbildung sichtbar.

Die Verteilung von Sinnesepithel und indifferentem Epithel hat sich gegenüber den vorher besprochenen Stadien nicht wesentlich verändert. Der nun neu hinzugekommene Abschnitt des Geruchsorgans, der Choanengang verdankt seine Entstehung den Oberkieferfortsätzen und ist dementsprechend nur von indiffe-

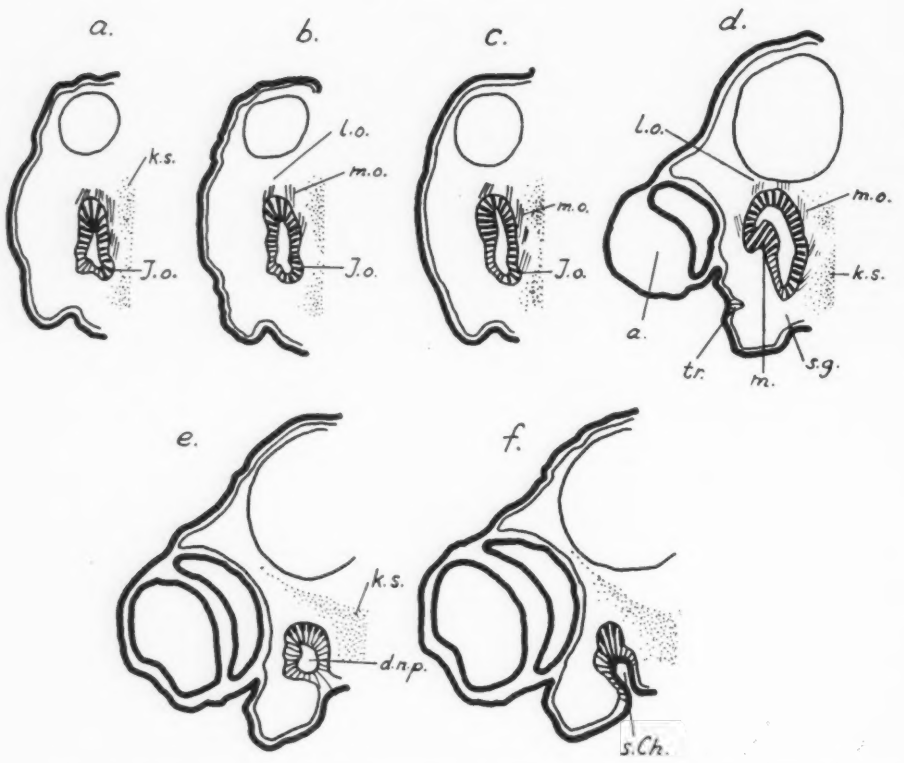

Abb. 10. *Emys europaea*. 3,5 mm Kopflänge. Ausgewählte Querschnitte a—f von oral nach caudal. Anlage des JACOBSONschen Organs (J.o.) und der Nasenmuschel (m); m.o. medialer Olfactorius; l.o. lateraler Olfactorius; k.s. knorpeliges Nasenseptum; a. Auge; tr. Anlage des Tränennasenganges; d.n.p. Ductus Nasopharyngicus; s.g. sekundärer Gaumen. 48fach.

rentem Epithel ausgekleidet. Olfactorisches und respiratorisches Epithel sind in ihrer histologischen Beschaffenheit nicht ohne weiteres abzugrenzen. Zwischen beiden besteht nämlich eine "Übergangszone", wie ich sie nennen möchte, deren Zellen weder dem einen noch dem anderen Abschnitt eindeutig zugeordnet werden können. Diese Übergangszone ist ungefähr im mittleren Drittel der lateralen Wand besonders stark ausgeprägt. Typisches olfactorisches Epithel ist nur im Dach der Nasenhöhle, also im Bereich der eigentlichen "pars olfactoria" deutlich feststellbar. Dieses olfactorische Epithel ist aus typischen Sinneszellen mit mehrreihig angeordneten, dichtgedrängten und der Basis des Epithels genäherten Kernen aufgebaut. Lumenwärts entsteht so ein kernfreier Abschnitt, dessen Höhe in der lateralen und medialen Wand allmählich abnimmt. Dagegen erscheinen im niedrigerem, indifferenten respiratorischen Epithel die Kerne gleichmässig verteilt und mehr der Zelloberfläche genähert oder sogar dieser anliegend. In der lateralen und medialen Wand der Nasenhöhle weist das Epithel

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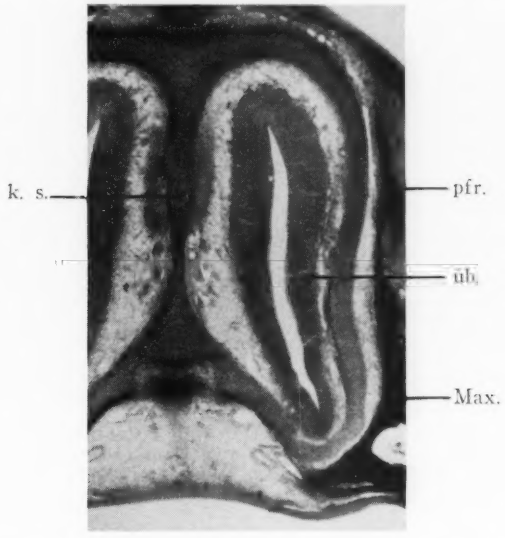

Abb. 11. *Emys europaea*. 7,5 mm Kopflänge. Querschnitte durch die Nasenhöhle; k.s. knorpeliges Nasenseptum; pfr. Praefrontale; üb. Übergangsteil; Max. Maxillare. Leitz Ortholux Apochromat 8 mm; Photookular 8fach.

eine weitgehende histologische Übereinstimmung auf. Daß jedoch auch in der medialen Wand eine "Übergangszone" vorhanden ist, kann daran festgestellt werden, daß diese Zellen reichlich von Fasern des Olfactorius versorgt werden. Dies ist bei der "Übergansgzone" der lateralen Wand nicht der Fall. Die geschilderte Struktur der Schleimhaut ändert sich, von einigen Einzelheiten abgesehen, nicht bis zu den ältesten Embryonen.

Im Gegensatz zu der eben geschilderten Beschaffenheit der Schleimhaut bei *Emys europaea* kann bei einem älteren *Natrix*-Embryo schon bei geringer Vergrösserung eindeutig eine scharfe Grenze zwischem olfactorischem und respiratorischem Epithel festgestellt werden. Die Abb. 11 u. 12 sollen einen Eindruck dieser Verhältnisse vermitteln.

Die Abb. 11 stellt einen Querschnitt durch die Nasenhöhle eines Embryo von *Emys europaea* mit 7,5 mm Kopflänge dar, die Abb. 12 die identischen Verhältnisse bei einem *Natrix*-Embryo von 8 mm Kopflänge. Für adulte Schildkröten haben sowohl FUCHS (1915) als auch MIHALKOVICS (1899) festgestellt, daß das Epithel der Nasenhöhle histologisch nur wenig differenziert ist und die einzelnen Abschnitte desselben sich nur durch wechselnde Höhe ihrer Zellen unterscheiden.

In dem Stadium von 3,5 mm Kopflänge sind die beiden Äste des Olfactorius deutlich zu unterschieden und bis zu ihrer Vereinigung und ihrem Eintritt in den Bulbus olfactorius zu verfolgen. Der mediale Olfactorius-Ast innerviert mit seinen Verzweigungen die ganze mediale Wand bis zum Boden der Nasenhöhle, also auch das JACOBSONsche Organ, während sich der laterale Ast nur bis in

Abb. 12. *Natrix natrix*. 8,0 mm Kopflänge. Querschnitt durch die linke Nasenhöhle. O. E. olfactorisches Epithel; r.E. respiratorisches Epithel. Weitere Angaben wie Abb. 11.

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die Höhe der Muschelanlage erstreckt. Diese Verhältnisse stützen unsere bisherigen Befunde über die Abschnitte der Nasenschleimhaut: das JACOBSONsche Organ ist nunmehr auch durch seine Innervierung identifizierbar, während das Fehlen von Olfactorius-Fasern den ventralen Abschnitt der lateralen Wand als indifferentes, bzw. respiratorisches Epithel kennzeichnet.

Bemerkenswert ist an diesem Embryo weiterhin, daß sich vom Ektoderm der Körperoberfläche unterhalb des Auges in Höhe der Muschelanlage eine Epithelleiste abzugliedern beginnt, deren Lage mit der undeutlich gewordenen Grenze zwischen Oberkieferfortsatz und äusserem Nasenfortsatz übereinstimmt. Diese Epithelleiste ist die Anlage des Tränennasenganges (Abb. 10 d). Auch dieser wird demnach bei *Emys* ebenso wie die Muschel und das JACOBSONsche Organ ontogenetisch sehr spät angelegt.

Von der knorpeligen Nasenkapsel ist das Septum in Gestalt zweier Knorpelstreifen angelegt, einem dorsalen in Höhe des Daches der Nasenhöhle und einem ventralen, der cranial unter den Boden der Nasenhöhle reicht. Diese beiden Knorpelstreifen vereinigen sich caudal im Bereich der Muschel und strahlen als einheitliche Masse zwischen den Choanengängen in das Septum interorbitale ein. Bis auf geringe Abweichungen entspricht dieses Stadium dem Embryo IV von *Chrysemys picta* mit einer Schildlänge von 4 mm von SEYDEL.

Das Auge ist weiter differenziert. In der Retina sind plexiforme und Körnerschichten deutlich erkennbar.

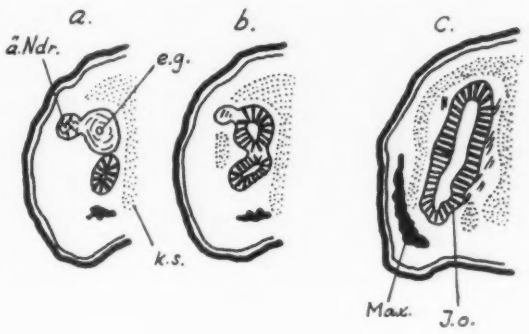

Abb. 13. *Emys europaea.* Kopflänge 5 mm. Angewählte Querschnitte (a—c) von oral nach caudal Bildung der äußeren Nasendrüse (ä.Ndr.) und Anlage des JACOBSONschen Organs (J.o.). e.g. Einführungsgang (Aditus nasi); Max. Maxillare. 48fach.

Embryo von Emys europaea mit einer Kopflänge von 5 mm. (Abb. 13).

Die untersuchte Querschnittserie zeigt, daß dieser Embryo in der Entwicklung weiter fortgeschritten ist. Der Einführungsgang wird von schmalen spindelförmigen Zellen ausgekleidet. Sie sind konzentrisch angeordnet und stellenweise dicht ineinandergekeilt und umhüllen das Lumen des Ganges manchmal so eng, daß sie es fast zu verschließen scheinen. An der Übergangstelle zwischen Einführungsgang und Haupthöhle bildet das Epithel eine lateral in das Mesoderm gerichtete Knospe (Abb. 13 a). Nach der Lage zu schliessen handelt es sich um die in Bildung begriffene äussere Nasendrüse *(Glandula nasalis externa)*. Die Zellen sind hier nicht spindelförmig, wie im angrenzenden Epithel, sondern mehr gerundet und schliessen lückenlos aneinander.

Das Epithel des Einführungsganges ist gegenüber dem anschließenden Epithel des Nasenschlauches noch nicht scharf abgegrenzt. Der caudale Abschnitt des Einführungsganges jedoch ragt bereits trichterförmig in die Nasenhöhle vor, wie dies in noch stärkerem Maße für das adulte Tier kennzeichnend ist. Der senkrecht stehende Hohlraum der Haupthöhle ist fast überall sehr eng und spaltförmig. Auf Abb. 13 c ist das JACOBSONsche Organ als schwache Rinne in der medialen Wand sichtbar, jedoch ist im Vergleich mit den jüngeren Stadien eine Verschiebung des Organs eingetreten: es liegt nicht mehr am Übergang zwischen dem Boden und der medialen Wand der Nasenhöhle, sondern mehr nach dorsal verlagert.

Dieses Stadium ist mit dem von SEYDEL beschriebenen Embryo V von *Chrysemys picta* mit 5 mm Schildlänge vergleichbar.

SEYDEL schreibt bei diesem Embryo über das JACOBSONsche Organ folgendes: "Das Sinnesepithel des JACOBSONschen Organs hat sich in der ganzen Länge des letzteren über den Boden der pars respiratoria hinweg auf die laterale Wand ausgedehnt. Diese Ausbreitung ist auf Kosten des indifferenten Epithels der lateralen Wand erfolgt; letzteres ist nunmehr auf einen schmalen Streifen reduziert, der zwischen der unteren Grenze der Riechschleimhaut und der oberen Grenze des Sinnesepithels des JACOBSONschen Organs eingeschoben ist. In der regionalen Ausbreitung des letzteren erinnert dieser Entwicklungs-

zustand bereits an die definitiven Verhältnisse. Doch ist das spezifische Epithel des Organes durchaus einheitlich" (SEYDEL l.c. p. 472).

Zweifellos hat also SEYDEL auch die cranial vom eigentlichen JACOBSONschen Organ gelegen Schleimhautpartien für Abschnitte des JACOBSONschen Organs gehalten. Wenn man aber die vordersten Bezirke der Nasenschleimhaut in diesem Stadium als Abschnitt eines JACOBSONsche Organs auffaßt, müßte man konsequenterweise u. E. auch bei jüngeren Embryonen so vorgehen und alle cranial von der Anlage des JACOBSONschen Organs gelegenen Sinnesepithelbezirke, letzlich auch die Rostralbucht gegen das Mesoderm, als Bezirke des JACOBSONschen Organs auffassen. Dies ist jedoch entwicklungsgeschichtlich sicherlich nicht richtig. Ganz besonders widerspricht dieser Ansicht jedoch die Versorgung des Bodens der Nasenhöhle durch den medialen Ast des Olfactorius. Die medialen Olfactoriusfasern greifen nicht, wie SEYDEL behauptet, auf die laterale Wand über. Ich halte daher die cranial vom JACOBSONschen Organ liegenden Abschnitte des Sinnesepithels, die von anderen olfactorischen Fasern innerviert werden, nicht zum JACOBSONschen Organ gehörig.

Die übrigen Veränderungen in der Nasenschleimhaut betreffen vor allem die völlige Reduktion der Muschelanlage und neu auftretende Reliefbildungen in der Nasenhöhle, von denen besonders eine ungefähr in halber Höhe der lateralen Wand vorspringende Epithelleiste zu erwähnen ist. Sie beginnt dicht hinter dem Einführungsgang, neigt sich nach caudal und ventral und verstreicht dann allmählich in der lateralen Wand. Es ist dies wahrscheinlich die von SEYDEL als "laterale Grenzfalte" bezeichnete Leiste der Schleimhaut. Sie bildet nach unseren Befunden jedoch keine genau Grenze zwischen dem respiratorischen und indifferenten Epithel, sondern liegt in der "Übergangszone" zwischen den beiden Epithelien.

Der Ductus nasopharyngicus beginnt nicht mehr direkt am caudalen Ende der Haupthöhle, sondern im letzten Drittel ihres Bodenteiles. Das Cavum nasi reicht daher dorsal über die Mündungsstelle hinaus. Diese Verhältnisse sind den beim erwachsenen Tier anzutreffenden bereits weitgehend ähnlich.

Von der knorpeligen Nasenkapsel ist außer dem bereits zu einer einheitlichen Masse verschmolzenem Septum noch das Tectum hinter der äusseren Nasendrüse vorhanden, welches bis in die Höhe der Mündung des Nasenrachenganges zieht. Fast über den gleichen Bereich erstrecken sich die seitlichen Wände der knorpeligen Nasenkapsel. Vom Nasenboden, dem Solum nasi, sind nur zwei isoliert liegende Knorpelelemente vorhanden: eines unterhalb des Einführungsganges und ein zweites, schmäleres, entlang des Septums.

Von den Deckknochen der Ethmoidalregion ist das Maxillare und Praemaxillare bereits angelegt.

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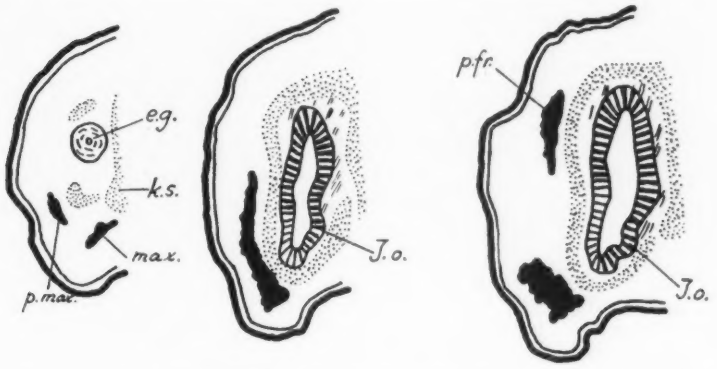

Abb. 14. *Emys europaea.* Kopflänge 6 mm. Ausgewählte Querschnitte (a—c von oral nach caudal). p.Max. Praemaxillare; Max. Maxillare; k.s. knorpeliges Nasenseptum; e.g. Einführungsgang; JACOBSONsches Organ (J.o.); pfr. Praefrontale. 48fach.

Embryo von Emys europaea mit einer Kopflänge von 6,0 mm. (Abb.14).

Das Epithel des Einführungsganges hat sich zu einem geschichteten Plattenepithel differenziert und gegen das angrenzende Sinnesepithel scharf abgesetzt. Das trichterförmige Endstück ragt weiter als im vorigen Stadium in die Haupthöhle vor. Die äußere Nasendrüse ist bereits deutlich als eine in einzelne Lappen gegliederte, tubulöse Drüse erkennbar.

In der Ausbildung der Schleimhaut stimmt dieses Stadium weitgehend mit dem von SEYDEL beschriebenen Embryo von *Emys lutaria* mit einer Schildlänge von 10 mm überein. Eine starke Vorwölbung der lateralen Wand bezeichnet SEYDEL als "Grenzwall". Er tritt offenbar in der Entwicklung nur vorübergehend auf und ist beim adulten Tier nicht mehr nachweisbar. Wir bezweifeln daher die Berechtigung, diesem Gebilde einen besonderen Wert beizumessen und es mit einem eigenen Namen zu versehen.

In diesem Stadium zeigt sich besonders deutlich der Unterschied der von SEYDEL gegebenen und von uns vertretenen Deutung. Nach unseren Befunden läßt sich auch in diesem Stadium keine Gliederung in der pars respiratoria erkennen. Dagegen unterscheidet SEYDEL in seiner Beschreibung und seinen Abbildungen hier 3 Abschnitte als JACOBSONsches Organ, die er durch schmale Streifen indifferenten Epithels trennt. Als echtes JACOBSONsches Organ ist jedoch auch in diesem Stadium nur diejenige Rinne in der medialen Wand zu bezeichnen, welche gegen den Ductus nasopharyngicus zieht. Eine weitere Stütze unserer Anschauung ist die Beziehung dieses Epithels zum medialen Ast des Olfactorius. Er innerviert die ganze mediale Wand, greift jedoch nicht unter dem Boden der Nasenhöhle auf die laterale Wand über.

Sehr ähnlich den adulten Verhältnissen ist der Aufbau der knorpeligen Nasenkapsel. Ihr Boden ist bereits weitgehend mit der seitlichen Wandung

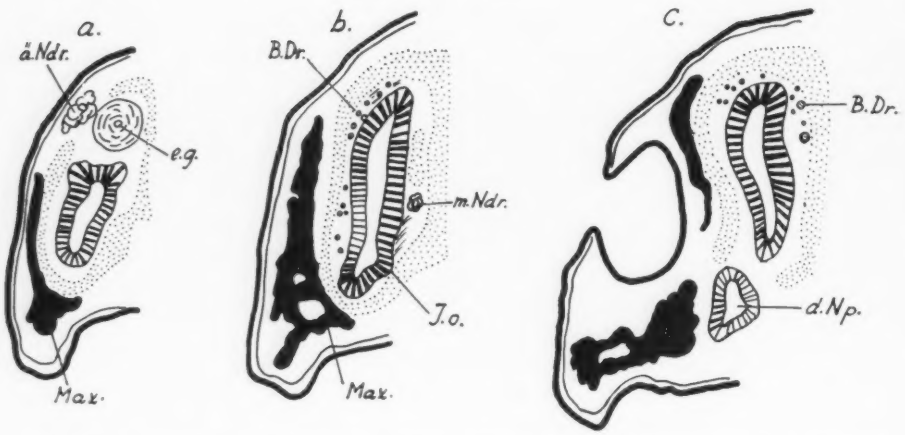

Abb. 15. *Emys europaea*. 7,5 mm Kopflänge. Ältester Embryo. Querschnitte a—c von oral nach caudal. ä.N.Dr. äußerer Nasendrüse außerhalb der Knorpelkapsel; Einführungsganges (e.g.); m.N.Dr. mediale Nasendrüse; J.o. JACOBSONsches Organ; Max. Maxillare; d.Np. Nasenrachengang; B.Dr. BOWMANsche Drüsen. 48fach.

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verschmolzen. Die im vorigen Stadium getrennten, dem Septum nasi benachbarten und cranial davon gelegenen Knorpelstreifen haben sich miteinander vereinigt. Nur im caudalen Abschnitt bleibt beiderseits des Septums und zwischen den Nasenrachengängen ein unpaarer, caudal gerichteter Fortsatz: die cartilago paraseptalis. Seine Entstehung steht nicht, wie ursprünglich vermutet wurde und in der Literatur angegeben wird, mit der Genese des JACOBSONschen Organs in einem Zusammenhang. Die früher häufig gebrauchte Bezeichnung "JACOBSONscher Knorpel" ist daher unrichtig und besser zu vermeiden.

Eine weitere beachtliche Veränderung in der Ethmoidalregion betrifft das zwischen Schleimhaut und knorpeliger Nasenkapsel gelegene Gewebe, die Submucosa. Während sie bei den jüngeren Embryonen vom übrigen Mesoderm des Vorderkopfes nicht zu unterscheiden war, zeigt sie sich nun durch die Bildung von Hohlräumen zwischen den Bindegewebszellen an: die Submucosa wird cavernös.

Praemaxillare, Maxillare und Frontale sind gegenüber den vorher beschriebenen Stadien vergrößert.

Embryo von Emys europaea mit einer Kopflänge von 7,5 mm. (Abb. 15).

Bei der Untersuchung dieses Embryos, des ältesten unserer Serie, zeigt sich ähnlich wie bei dem vorher beschriebenen, der Unterschied zwischen unseren Befunden und ihrer Deutung gegenüber der Arbeit von SEYDEL: das JACOBSONsche Organ nimmt auch hier bei weitem nicht einen so großen Teil der Wand der Nasenhöhle ein, wie sie von SEYDEL angegeben wird.

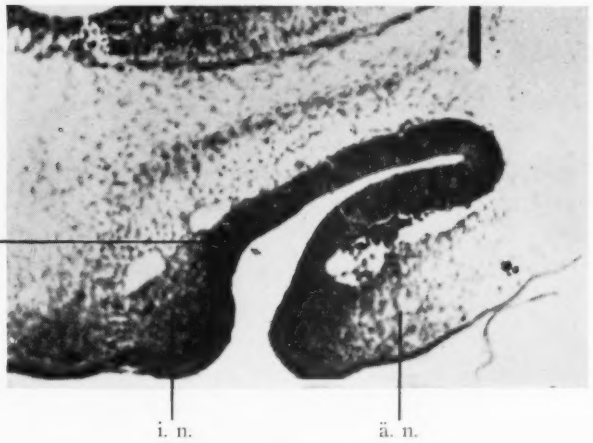

Abb. 16. *Emys europaea*. Kopflänge 2,5 mm. Erste Anlage des JACOBSONschen Organs (J.o.); ä.n. äußerer Nasenfortsatz; i.n. innerer Nasenfortsatz; Leitz Ortholux, Apochromat 8 mm, Photookular 8fach.

Der Einführungsgang ist gegenüber dem letzten beschriebenen Stadium deutlich verlängert, ragt verhältnismäßig weit in die Haupthöhle und zeigt ein erweitertes kreisrundes Lumen. Die laterale Nasendrüse liegt nun zum größten Teil außerhalb der knorpeligen Nasenkapsel und ist teils dem Tectum und der Seitenwand aufgelagert. Der Querdurchmesser der Haupthöhle ist breiter geworden.

In der Nasenschleimhaut ist außer der bereits in den vorigen Stadien erwähnten Übergangszone zwischen respiratorischem und olfactorischem Epithel an der lateralen Wand nun auch eine solche am Boden der Nasenhöhle deutlich zu erkennen. Das JACOBSONsche Organ besitzt noch immer die gleiche Lage wie im vorigen Stadium, seine Rinne hat sich jedoch, besonders im rostralen Bereich, noch etwas vertieft. Die Abb. 17 zeigt einen Querschnitt durch den ventralen Teil der Nasenhöhle eines Embryos von 7,5 mm Kopflänge. Hier ist die tiefste Stelle der Eindellung im JACOBSONschen Organ sichtbar. Als Vergleich ist auf Abb. 16 die erste Anlage des Organs bei einem Embryo von 2,5 mm Kopflänge abgebildet.

Dieser älteste von uns untersuchte Embryo stimmt mit dem von SEYDEL ebenfalls als ältestem Stadium beschriebenen Embryo von *Emys lutaria* mit einer Schildlänge von 14 mm überein. Die Gestalt und grundsätzliche Form der Nasenhöhle auf SEYDELS Abbildungen stimmt ebenfalls mit unseren Befunden weitgehend überein, nicht jedoch die von ihm angegebene Verteilung der einzelnen Epithelien. Nach SEYDEL müßten dorsal und ventral vom JACOBSONschen Organ, sowie in der lateralen Wand schmale Streifen von indifferentem Epithel vorhanden sein. Dies ist jedoch auf unseren Präparaten nicht feststellbar. Dazu kommt, daß auch in diesem Stadium der mediale Ast des Olfactorius mit seinen Verzweigungen das JACOBSONsche Organ und einen kleinen Abschnitt der Schleimhaut

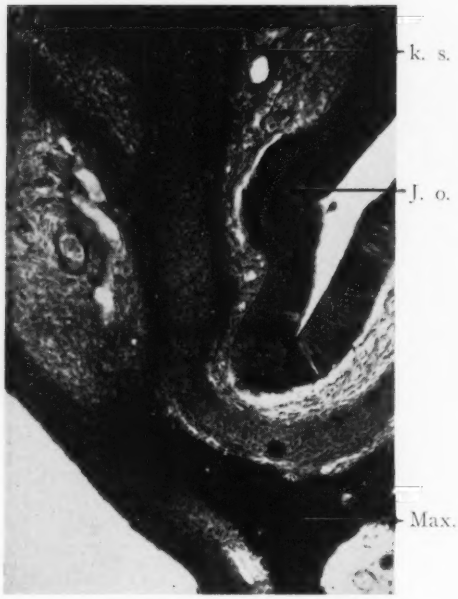

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Abb. 17. *Emys curopaea*. 7,5 mm Kopflänge. Querschnitt durch die Nasenhöhle mit der tiefsten Stelle der Eindellung des JACOBSONschen Organs (J.o.); k.s. knorpeliges Nasenseptum; Max. Maxillare. Leitz Ortholux, Apochromat 8mm, Photookular 8fach.

ventral davon innerviert, nicht aber, wie es SEYDEL angibt, unter dem Boden der Nasenhöhle hindurchzieht und auch Teile der lateralen Wand versorgt.

Die Submucosa zeigt in diesem Stadium bereits deutliche Blutgefäße, die besonders in der Nähe des Nasenseptums angehäuft sind. Außer den Zweigen des Olfactorius finden sich noch einige Ästchen des Ramus lateralis trigemini in der Submucosa. Nur im ventralen Teil der Nasenhöhle, unterhalb des Tectum nasi, sind diese Fasern genauer zu verfolgen.

Die mediale Nasendrüse ist erstmals nachweisbar: sie liegt rostralwärts in einer vom knorpeligen Nasenseptum gebildeten Rinne und erstreckt sich etwa von der Höhe des Einführungsganges bis zu dem von SEYDEL beschriebenen "Stützknorpel der medialen Grenzfurche" (Abb. 15 b). Die Drüsenanlage ist natürlich noch wenig entwickelt und differenziert und es ist kein Ausführungsgang in die Nasenhöhle nachweisbar. Die den Drüsenkörper aufbauenden Zellen gleichen denjenigen der äußeren Nasendrüsen, wie wir sie bei einem Embryo von 5 mm Kopflänge beschrieben haben. Die mediale Nasendrüse wird nach unserem Befund also in einem späteren Entwicklungsstadium als die laterale Nasendrüse angelegt. In der Submucosa liegen verschiedentlich BOWMANsche Drüsen (Abb. 15 b, c), die besonders im Dach der Nasenhöhle in der regio olfactoria angehäuft sind, jedoch in ventralen Abschnitt der medialen

Wand, also im Bereich des JACOBSONschen Organs fehlen, wie bereits SEYDEL hervorgehoben hat. Die BOWMANschen Drüsen sind kugelig, birnförmig oder von gestreckter Gestalt und aus einer nur geringen Anzahl von Zellen aufgebaut. Die Ausführungsgänge, die sich zwischen den Drüsenzellen befinden, konnten nur auf einigen Querschnitten mit Sicherheit nachgewiesen werden.

Die knorpelige Nasenkapsel ist vollkommen ausgebildet. Sie hat zwischen der hinteren Grenze des Einführungsganges und dem Choanengang die Gestalt eines weitlumigen, die Haupthöhle umschliessenden Rohres. Nur in ihrem Boden befindet sich beiderseits vom Septum im Bereiche des caudalen Abschnittes des JACOBSONschen Organs und vor den Choanengängen eine Öffnung, das Foramen praepalatinum. Rostralwärts ist die Nasenkapsel an der Oberseite weit offen und umhüllt ventral den Einführungsgang. Nach caudal setzt sich der Boden der Nasenkapsel als ein dem Septum eng anliegender Knorpelstreifen fort: Cartilago paraseptalis. Etwas über der Basis des Septums ausstrahlend und in die Submucosa parallel zum Septum einragend ist ebenfalls ein Knorpelstreifen vorhanden, der in der Höhe des Einführungsganges beginnt. Er nimmt dort die mediale Nasendrüse auf, löst sich weiter caudal von seinem Zusammenhang und kommt frei in der Submucosa zu liegen. Dieser Knorpel ist bei dem hier untersuchten Embryo weitaus stärker ausgebildet als bei dem ältesten von SEYDEL beschriebenen Keimling. SEYDEL hat, wie erwähnt, diesen Knorpel als Stützelement der medialen Grenzfalte bezeichnet. Diese mediale Grenzfalte ist bei dem hier beschreibenen Embryo nur sehr schwach ausgebildet. Auch bei adulten Emys ist diese mediale Grenzfalte nicht immer gleich stark ausgebildet, wie ein Vergleich der Abbildungen von SEYDEL und MIHALKOVICS lehrt. Die mediale Grenzfalte bildet sich nach unseren Befunden erst nach der Ausbildung des genannten Knorpels in ihrem vollen Umfang aus. Wir halten es daher nicht für richtig, wenn SEYDEL annimmt, daß der Knorpel die Faltenbildung der Schleimhaut verursachen würde.

Die von SEYDEL in der seitlichen Wand der Nasenkapsel beschriebenen beiden "Nervenlöcher" konnten wir in unseren Serien nicht finden. SEYDEL macht übrigens auch keine weiteren Angaben darüber, welche Nerven durch diese Foramina hindurchtreten sollten.

Der Nasenrachengang der adulten Sumpfschildkröten weist nach den Angaben von SEYDEL einen Recessus auf, der als Falte bis zum caudalen Abschnitt der Haupthöhle reicht. Diesen Recessus konnte ich dem ältesten von uns untersuchten Embryo nicht auffinden.

Die Verknöcherungen von Maxillare, Praemaxillare und Praesphenoid sind weiter fortgeschritten. Als neue Ossifikation kommt die der Vomeres, die auf Querschnitten zwischen den Choanen erkennbar sind, hinzu. Die Form des Auges und der histologische Bau von Retina, Pigmentepithel, Chorioidea und Sclera stimmen mit der Ausbildung beim adulten Tier weitgehend überein. Augenlider sind deutlich vorhanden.

Das älteste von uns untersuchte Entwicklungsstadium unterscheidet sich im Bau des Geruchsorgans und des JACOBSONschen Organes von der Adultform durch folgende wesentliche Merkmale: 1. Verringerung des Querdurchmessers

der Haupthöhle; 2. geringe Entwicklung der medialen Nasendrüsen; 3. Fehlen eines Recessus des Choanenganges; Das JACOBSONsche Organ ist nach unseren Befunden eine Rinne, die im medialen Teil des Epithels der Nasenwand liegt und u.a. auf Grund seiner Innervierung identifiziert werden kann. Die SEYDELschen Angaben über die Gliederung des von ihm etwas anders lokalisierten JACOBSONschen Organs in drei, durch indifferentes Epithel getrennte Sinnesepithelabschnitte können nicht bestätigt werden.

III. DISKUSSION

Den hier vorgelegten Befunden bezüglich des JACOBSONschen Organs von *Emys europaea* schliesse ich hier eine kurze Stellungsnahme zu den Ergebnissen anderer Untersucher an. MIHALKOVICS (1899) fand bei der Untersuchung der Schleimhaut einer erwachsenen *Emys sp.* einen Gang in der medialen Wand, der etwas höher als die von uns beschreibene Rinne liegt, weiter caudal mit der umgebenden Submucosa in Verbindung tritt und dort den Ausführungsgang der medialen Nasendrüse aufnimmt. Er nimmt an, daß dieser Gang einem JACOBSONschen Organ entspricht, seine Beobachtung konnte jedoch bisher von keinem anderen Untersucher bestätigt werden. Auch ich halte eine Identifizierung mit einem JACOBSONschen Organ für wenig gesichert. Man könnte zwar annehmen, daß das JACOBSONsche Organ bei den Testudinaten als Rudiment nicht immer gleichmäßig ausgebildet wird. Es könnte sich auch eine Rinne als Anlage eines JACOBSONschen Organs in ihrem tiefsten rostralen Bereich zu einem Rohr schließen. Dieses Verhalten würde vielleicht die Angaben von MIHALKOVICS erklären. Der beschriebene Gang in der Schleimhaut könnte aber auch der Ausführungsgang der medialen Nasendrüse sein, die wegen ihrer engen Beziehung zum JACOBSONschen Organ häufig als JACOBSONsche Drüse bezeichnet wurde.

MCCOTTER (1917) hält hingegen im Gegensatz zu MIHALKOVICS die SEYDELschen Befunde insofern für berechtigt, als er bei *Chrysemys* ebenfalls mehrere Abschnitte im Sinnesepithel der vorderen Haupthöhle zu unterscheiden vermeint. Er unterscheidet nicht nur 3 Abschnitte wie SEYDEL, sondern sogar vier, die wie bei SEYDEL ebenfalls durch schmale Streifen indifferenten Epithels voneinander getrennt seien. Da er aber keine embryologischen Befunde als Stütze seiner Ansicht vorbringen kann und diese allein, wie unsere Untersuchung zeigte, für die Klärung in Frage kommen, muß seine Identifizierung dieses Bezirkes mit einem JACOBSONschen Organ ebenso wie die Ansicht von SEYDEL abgelehnt werden.

Interessant sind in diesem Zusammenhang die Ergebnisse von RÖSE (1893) der bei Krokodilembryonen feststellen konnte, daß ein JACOBSONsches Organ während der Entwicklung als Schleimhautfalte auftritt, die sich bis in den

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Nasenrachengang fortsetzt. Bei einigen Arten soll diese Rinne beim adulten Tier erhalten sein, bei anderen nur in der Ontogenie vorübergehend auftreten. Die Ergebnisse von Röse lassen sich mit der von uns vertretenen Ansicht weit besser in Übereinstimmung bringen, als diejenigen von Seydel und McCotter. Nach Röse besitzen auch die Krokodile keine Gliederung des Jacobsonschen Organs in mehrere Abschnitte, was mit unseren Befunden gut in Einklang stehen würde. Wir glauben, daß eine weitere Untersuchung der Crocodilier hier ebenfalls eine weitere Klärung ermöglichen wird.

Fuchs (1915) betont, daß Seydel bei seinen Untersuchungen ausschließlich die Amphibien zum Vergleich herangezogen habe, daß jedoch die Schildkröten zunächst innerhalb der Amnioten, deren Charakter sie entschieden tragen, verglichen werden müssen. Dies hatte sich bei Fuchs' Untersuchungen über die Gaumenbildung als unbedingt notwendig erwiesen. Er ging bei seinen Vergleichungen von dem zweifellos sehr primitiven Amnioten *Sphenodon* aus. In ähnlicher Weise, wie Fuchs bezüglich der Gaumenbildung, haben wir unsere vergleichende Untersuchung bewußt innerhalb der Amnioten ausgeführt.

Gegen die Ansicht, daß die Rinne in der medialen Wand der Schleimhaut einem Jacobsonschen Organ entspricht, spricht Matthes (1936) die Meinung aus, daß man dieses bei einer so geringen Selbständigkeit gegenüber der Haupthöhle nur schwerlich als ein "Organ" ansprechen kann. Das Jacobsonsche Organ hat sicherlich bei *Emys* und ähnlichen Formen physiologisch seine offenbar bei den Squamaten hochentwickelte Funktion eingebüßt. Dort dient es einer besonderen Chemoreception durch die Mundhöhle mittels besonderer Züngelbewegungen (Schlangen und Eidechsen); dies ist sicherlich bei Schildkröten nicht der Fall. Morphologisch ist jedoch grundsätzlich auch ein rudimentäres Organ mit einem voll funktionsfähig ausgebildeten vergleichbar.

Nach der hier gegebenen Darstellung der Entwicklungsgeschichte ist bei *Emys europaea* das Jacobsonsche Organ mit dem von Seydel als "pars superior" bezeichneten Abschnitt im vorderen Teil der Haupthöhle identisch. Nur dieser Sinnesepithelbezirk allein ist mit dem Jacobsonschen Organ anderer Sauropsiden homologisierbar. Ob dieses Jacobsonsche Organ, wie es hier bei *Emys europaea* beschrieben wurde, auch bei anderen Schildkröten vorhanden ist, muß offen bleiben. Einerseits ist die Entwicklungsgeschichte der Chelonier nur wenig oder überhaupt nicht bekannt, andererseits zeigen die bisherigen Untersuchungen von adulten Tieren innerhalb der Schildkröten bedeutende Unterschiede im Aufbau der Ethmoidalregion, die u.E. anzeigen, daß auch unsere Befunde bezüglich des Jacobsonschen Organs zunächst nicht verallgemeinert werden dürfen.

Eine phylogenetische Deutung des Jacobsonschen Organs müßte auf Grund unserer derzeitigen Kenntnisse dem Chelonier *Emys europaea* eine vermittelnde Stellung zuschreiben, die wie folgt gekennzeichnet werden kann: Das Organ besitzt bei den Squamaten seine stärkste Ausbildung und offen-

bar damit zusammenhängend die am höchsten differenzierteste Funktion (KAHMANN 1938); es erreicht eine weitgehende Selbständigkeit vom Nasenraum. Bei *Emys europaea* (und wahrscheinlich ähnlich bei den anderen Schildkröten) ist das Organ im Zusammenhang mit einer stark geminderten Funktionstüchtigkeit nur wenig ausgeprägt; es bleibt als Rinne innerhalb des gemeinsamen Nasenraumes in direktem räumlichen Kontakt mit dem eigentlichen Geruchsorgan, kann jedoch auf Grund der Entwicklung und der Innervation identifiziert werden. Bei Krokodilen und Vögeln ist es nur während der Entwicklung ähnlich wie bei *Emys europaea* nachweisbar, adult offenbar nicht mehr vorhanden und hat seine Funktion (im Sinne der Spezialleistung bei den Squamaten) vollkommen eingebüßt.

IV. ZUSAMMENFASSUNG

Die Entwicklung der Nasenhöhle und des JACOBSONschen Organs wurde bei zehn verschieden alten Embryonen der europäischen Sumpfschildkröte *(Emys europaea)* untersucht und mit anderen Sauropsiden verglichen.

Die erste Anlage und die frühesten Entwicklungsvorgänge des JACOBSONschen Organ stimmen bei *Emys europaea* mit anderen Reptilien überein. Das Organ tritt jedoch bei *Emys europaea* ontogenetisch deutlich später auf. Die zunächst bandförmige Anlage liegt in der medialen epithelialen Wand der Nasenhöhle, vergrößert sich und bildet eine mäßig tiefe Rinne. In dieser Form bleibt es beim erwachsenen Tier als Rudiment erhalten.

Die Muschel, der Tränennasengang und die mittlere Nasendrüse zeigen ebenso wie das JACOBSONsche Organ im Vergleich mit anderen Reptilien deutliche Merkmale der Rückbildung. Sie werden ontogenetisch ebenfalls relativ später angelegt. Muschel und Tränennasengang werden während der Entwicklung nur vorübergehend gebildet.

V. LITERATUR

Es werden hier nur die im Text zitierten Arbeiten angegeben. Bei der Abfassung der Arbeit wurde von einer historischen und allgemeinen Einleitung über das JACOBSONsche Organ und das Geruchsorgan abgesehen. Eine gute Zusammenfassung mit weiterführenden Literaturangaben findet man bei MATTHES (1934).

BEECKER, A., Vergleichende Stilistik der Nasenregion bei den Sauriern. Gegenbaur Morph. Jb. 31 (1903): 565—620.

BORN, G., Nasenhöhle und Tränennasengang der Amnioten. Gegenbaur Morph. Jb. 5 (1879): 62—141 u. 401—403.

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Born, G., Nasenhöhle und Tränennasengang der Amnioten. Gegenbaur Morph. Jb. 8 (1883): 188—233.

Cohn, F., Zur Entwicklungsgeschichte des Geruchsorgans des Hühnchens. Arch. mikr. Anat. 61 (1903): 133—151.

Fuchs, H., Untersuchungen über die Phylogenie und Ontogenie der Gaumenbildungen bei den Wirbeltieren. Ztschr. f. Morph. u. Anthropol. 10 und 11 (1907/1908).

— Über Bau und Entwicklung des Schädels von Chelone imbricata. In: A. Völtzkow, Reisen in Ostafrika. 1915.

Ganin, F., Einige Tatsachen über das Jacobsonsche Organ der Vögel. Zool. Anz. 13 (1890): 285—287.

Grewe, F. J., Nuwe gegewens aangaande die ontogenese van die neurskliere, die orgaan van Jacobson en die dekbene van die skedel by die genus anas. Ann. Univ. Stellenbosch, Jg. 27 (1951); Reeks A. No. 3.

Kahmann, H., Über das Jacobsonsche Organ der Echsen. Ztschr. f. vergl. Physiol. 26. (193): 669—696.

Matthes, M., Geruchsorgan. In: Handb. d. vergl. Anat. d. Wirbelt. von Bolk-Göppert-Kallius-Lubosch, Bd. II/2 (1934): 879—943.

McCotter, The vomero-nasal apparatus in Chrysemys punctata and Rana catesbyana. Anat. Rec. 13 (1917): 51—69.

Mihalkovics, V. v., Nasenhöhle und Jacobsonsches Organ. Eine morphologische Studie. Anat. Hefte 11 (1899).

Peter, K., Geruchsorgan und Jacobsonsches Organ. In: Hertwig, O., Handb. d. verg. u. exp. Entwicklungslehre der Wirbelt., Bd. II/2 (1899): 1—78.

Röse, C., Das rudimentäre Jacobsonsche Organ beim Krokodil und beim Menschen. Anat. Anz. 1893: 458—472.

Seydel, O.: Über die Nasenhöhle und das Jacobsonsche Organ der Sumpfschildkröten. Eine vergleichend anatomische Untersuchung. Festschr. 70. Geburtstag von C. Cegenbaur, Bd. 2 (1896).

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REPRODUCTIVE CYCLE OF THE MALE INDIAN GERBILLE TATERA INDICA CUVIERII *(WATERHOUSE)*

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India

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INTRODUCTION

The Rodentia exhibit a variety of reproductive patterns; some species show a continuous reproductive activity while the others exhibit a regular periodicity. Continuous reproductive activity throughout the year, with no period of anestrum, is seen in the grey squirrel *Sciurus carolinensis* (ALLANSON 1939), in *Sciurus vulgaris* (ROWLANDS 1938), in the prairie vole *Microtus ochragaster* (JAMESON Jr. 1947) and in the palm squirrel *Funambulus palmarum* (PRASAD 1951). From a consideration of the fertility of the females of the wild brown rat *Rattus norvegicus*, PERRY (1945) records that there is no seasonal variation in the breeding capacity of the females and it is likely that very few males survive to such an age that they become sexually inactive. Working on the same species of rat occurring in corn ricks and urban areas of England, LESLIE, VENABLES and VENABLES (1952) concluded that the rats bred throughout the

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year with a peak between the months January and June. Under laboratory conditions, the cotton rat *Sigmodon hispidus* has been shown by MEYER and MEYER (1944) to breed throughout the year.

On the other hand, a seasonal rhythm of intensity of reproductive activity has been noticed in *Marmota monax* (RASMUSSEN 1917), in the hedgehog, *Erinaceus europæus* (ALLANSON 1932), in *Citellus tridecemlineatus* (WELLS 1935) and in the bank vole *Clethrionomys* (ROWLANDS 1938). Cyclic reproductive activity has been noticed in *Rattus rattus* and *Rattus norvegicus* in the tropics by BUXTON (1936), who also describes the variation in the fertility of *Rattus rattus* depending on environmental changes and food supply. *Peromyscus maniculatus* (JAMESON Jr. 1953) commences to breed in late winter and reaches the peak of activity by April. Reproduction ceased in August in 1950 while sexual activity continued unabated throughout the year in 1949. The changes in the duration of the breeding seasons from year to year suggests that the factors controlling the span of reproduction are not entirely intrinsic but are to a large extent extrinsic.

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Cyclical reproductive phenomena are generally characteristic of mammals living in temperate and subarctic regions. In some of these the cold winter months are passed in a torpid state during which the reproductive activity ceases. Such extreme environmental variations do not occur in the tropical and subtropical regions, except under certain xerotic conditions where aestivation may occur similarly associated with cessation of breeding. Extensive and interesting investigations on the breeding seasons of animals in the tropical regions has been made by the Oxford University Expedition of 1933—34 to the northern New Hebrides (in the western Pacific ocean), where environmental conditions vary less during the year than on any other land surface of the world. The findings are published by BAKER and HARRISON (1936), BAKER and BAKER (1936), BAKER and BIRD (1936), BAKER, MARSHALL and HARRISON (1940) and BAKER (1947 a and b). The investigation attempted to determine whether in such a uniform climate the animals reproduce continuously or seasonally, and if breeding seasons occur, whether they could be correlated with environmental variations. Most of the plants and animals showed marked seasonal sexual activity. The fruit bat *Pteropus geddei,* the insectivorous bat *Miniopterus australis* and the passerine bird *Pachycephala pectoralis* had one breeding season in the year while the lizard *Emoia cyanura* bred throughout. MARSHALL (1947) has shown that the fruit bat *Pteropus giganteus* living in the stable climate of Ceylon has a single sharply defined breeding season. GOPALAKRISHNA (1949) has recorded a similar restricted breeding season in the bat *Scotophilus wroughtoni* from Bangalore.

MEASROACH (1954) described the reproduction in the females of two species of gerbilles from South Africa and shows that *Tatera brantsi* from Transvaal breeds all the year round but at a reduced rate from October to December while

Tatera afra from south west Cape has a definite breeding season from August till the end of March. No mention was made of the sexual activity in the male.

The present paper records the male sexual cycle in an Indian Cricetid rodent, the gerbille, *Tatera indica cuvierii* (WATERHOUSE), in which there appears to be a regular cycle of reproductive activity. It was observed (PRASAD 1954 a) that in this species the young are born from the last week of September to the first week of March and it was felt that a study of the male sexual cycle would offer points of interest.

MATERIAL AND METHODS

One hundred and twenty two male gerbilles were collected during the period, December 1952 to July 1954. They were obtained from the fields in the neighbourhood of Bangalore. The animals were scared out of their burrows and captured by net. A few burrow systems were dug out and their occupants captured. Live animals were brought to the laboratory and anaesthetised by ether or chloroform. The body weight and length were noted. The genital organs were then dissected free of fat and connective tissue and dropped entire into the fixative. The most common fixative used was Bouin's fluid. The material was transferred to 70% alcohol and later dissected out and the testes, epididymedes, seminal vesicles, Cowper's glands and penis weighed on a chemical balance. The testes and epididymedes were sectioned at 10 micra and stained in iron haematoxylin. The diameter of the seminiferous tubules was calculated as follows. Outline drawings of ten tubules, circular in outline, selected from the periphery of the section were made by means of a horizontal projection apparatus at a constant magnification of 100 diameters. The greatest width at right angles to the two axes of the tubules was taken as the diameter and the average of 20 diameters was calculated. In view of the difficulty of estimating the volume of the interstitial cells or their number, the sectional area of the cells was calculated from camera lucida drawings drawn at a magnification of 1600 diameters on millimeter graph paper. The diameter of the epididymis tubules was taken in the body region and includes the surrounding muscle layers. The presence of spermatozoa in the testes, epididymis and vas deferens was considered a sign of sexual maturity. The general arrangement of the reproductive organs of the male gerbille resembles that of the rat.

GROWTH AND BREEDING SEASON

Figure 1 is a scatter diagram of the body weights of all the males collected. It was observed in an earlier paper (PRASAD 1954 a) that the young gerbilles

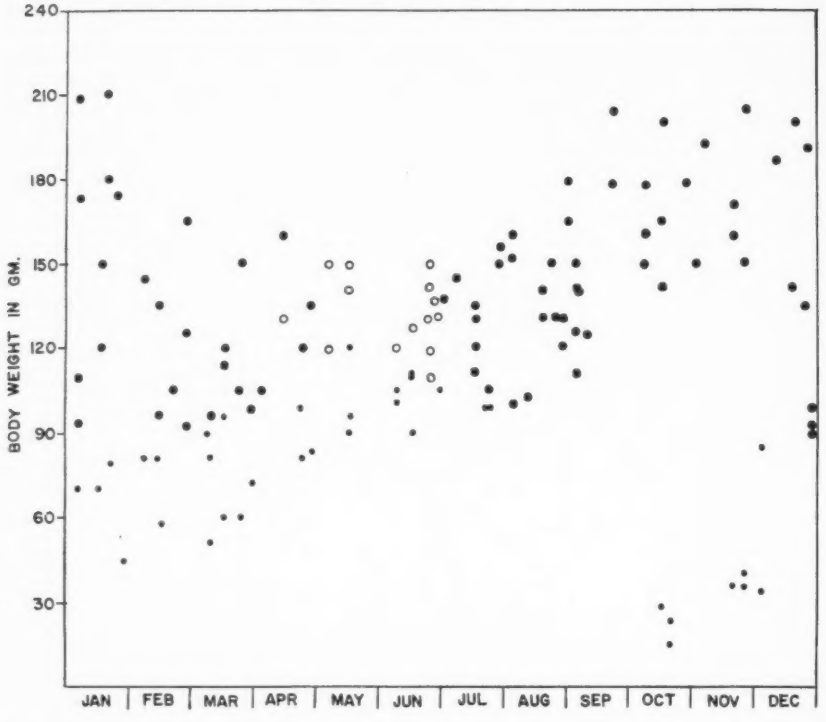

Fig. 1. Scatter diagram showing the body weight of all the animals collected and plotted on the dates of their collection.

● Mature animals with sperms in the testes.
o Mature animals without sperms in the testes. Animals in regression or quiescence.
• Immature animals.

(The same scheme is followed in all the scatter diagrams.)

are born during the months September through March. Hence, from the beginning of October to the middle of July next, the spread of the body weights is greater on account of the presence of young animals and maturing adults in the collection. From a study of the seasonal distribution of the body weights it is seen that the number of first year males with body weight greater than 75 grams is on the increase from December, and by the middle of June most of the animals of the first year group have reached a body weight of over 90 grams. Thus all males born either early or late in the breeding season attain a body weight of 90 grams or over by the next May or June. Twelve males collected in August all weighed over 100 grams. The weight gradually increases to a maximum of 210 grams in the breeding season. The lightest animal in which spermatozoa were found in the testes weighed 90 grams and was caught in the last week of December. The appearance of such animals in the middle of the breeding season shows that young males born earliest in the breeding season

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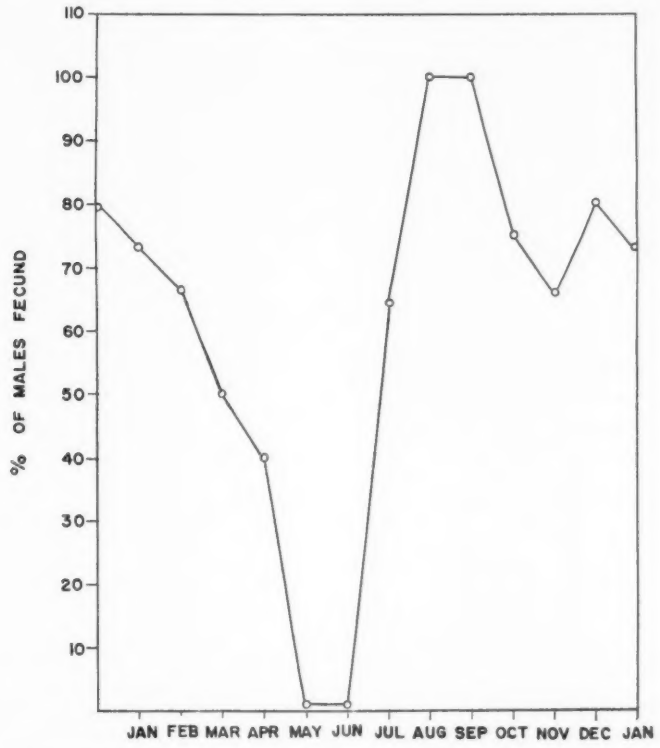

Fig. 2. Graph showing the monthly percentage of animals with spermatozoa in the testes. The figures include the average of the mature and immature animals.

namely in the middle of September, may become sexually mature in the same breeding season in which they were born, i.e. by December. Such animals continue to be active in a subdued state during the first summer of their birth-May and June, till the commencement of breeding season in July. Activity in the spermatogonial cells is greater in these animals than in those of the other age groups. Sperm production ceases completely in all animals during the period of regression in May and June.

Activity of the males as determined by the presence of sperms in the testes, epididymides and vasa deferentia (Table 1 and Fig. 2) extends from July to the end of April. Animals caught in the months of May and June include those which have been previously active and are now in a state of quiescence, as well as immature animals brought forth comparatively late in the breeding season. The distribution of the body weights in these months shows that animals of the heavier weight groups of the previous season either suffer heavy mortality or undergo considerable reduction in body weight. The ranges in body weights

of pre- and post-pubertal animals overlap during this period. The reduction in body weight closely follows the cessation of activity in the testes.

TABLE I. Number of gerbilles with and without spermatozoa in the testes obtained in each month.

Month.	Total number of gerbilles examined	Numbers of animals with spermatozoa	Number of animals without spermatozoa	% of gerbilles with spermatozoa
January	11	8	3	72.7
February	9	6	3	66.6
March	14	7	7	50.0
April	9	4	5	40.0
May	7	0	7	00.0
June	12	0	12	00.0
July	10	8	2	63.7
August	12	12	0	100.0
September	11	11	0	100.0
October	8	6	2	75.0
November	9	6	3	66.6
December	10	8	2	80.0

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CHANGES IN THE TESTES

Size of the testes: — There is a considerable variation in the size of the testes in the different seasons of the year. The average length and breadth of the two testes range from 26 × 15 mm in the active to 15 × 10 mm in the regressed state. The size increases gradually from the middle of July, reaches the maximum by the middle of August and continues maximal till the end of the breeding season. A sharp fall in size is registered at the beginning of April and continues through the month to reach a culmination in May and June. At this time there is considerable variation in testes sizes dependent on the state of regression or on the growth of the testes in immature animals.

Fig. 3 is a scatter diagram of the combined weight of the two testes. In the months of May and June the average weight of the testes of the adults is 0.75 grams as compared with 0.9 grams of the testes of immature animals. The weight of the testes of immature animals either corresponds with or is slightly more than that of the adults in regression. The weight increases rapidly from the middle of July and reaches a maximum of 6.75 grams by the middle of September. The testes weight of the sexually active adults is maintained at a relatively high level (average weight 4.5 grams) throughout the breeding season. The spread of the weights is, however, great in this period on account of individual differences in growth. As the breeding season draws to a close there is a

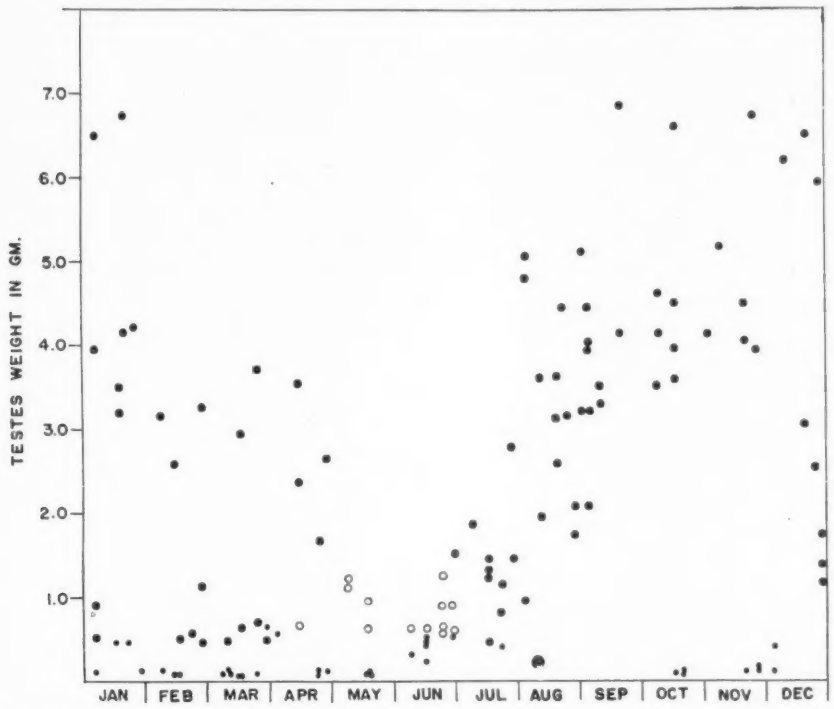

Fig. 3. Scatter diagram of the weight of the testes showing the variation in weight in the different months.

gradual reduction in weight commencing from the end of March. During the peak of regression in May and June the increasing testes weights of immature animals overlap the downward trend of testes weights of adults in regression (see appendix 1).

The increase in weight of the testes is due to:—

1) increase in diameter of the seminiferous tubules consequent on increased activity of the seminiferous epithelium.
2) increase in length of the seminiferous tubules.
3) increase in the size of the interstitial cells.

Diameter of the seminiferous tubules:— It has been shown in a number of animals that the diameter of the seminiferous tubules increases greatly concurrent with the increase in spermatogenetic activity in the breeding season. In the gerbille, the tubule diameter varies from about 90 micra in the period of regression in May and June to a maximum of 190 micra at the height of the breeding season. Figure 4 shows the seasonal variation in the diameter of the tubules. In juvenile animals tubule diameter increases from 45 micra at the

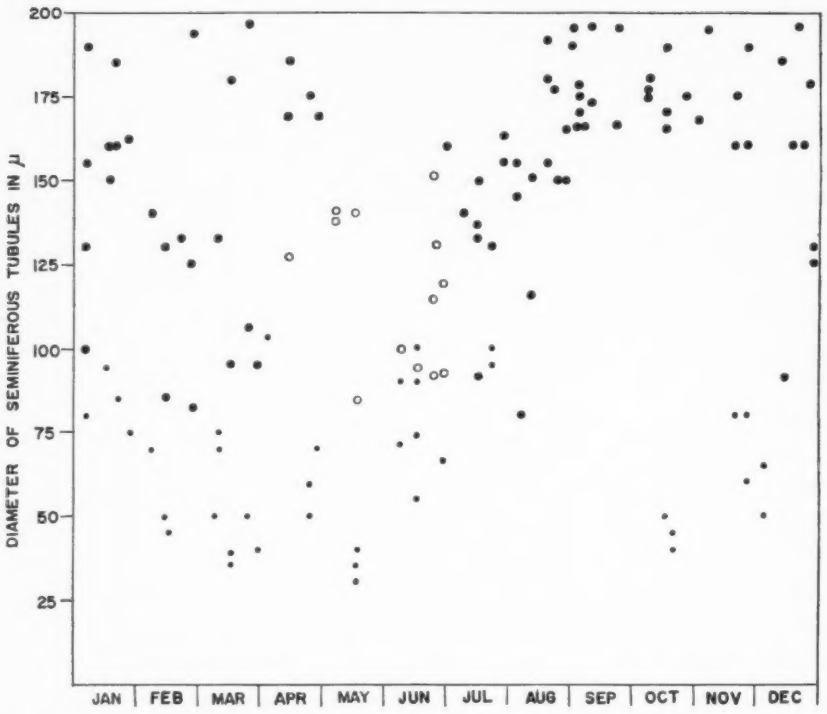

Fig. 4. Scatter diagram showing the seasonal variation of the diameter of the seminiferous tubules.

beginning of October to 90 micra by the last week of December when the production of spermatozoa begins.

On the basis of the relation between the diameter of the seminiferous tubules and testes weight the animals can be classified into four different groups:—

a) immature animals coming for their first breeding season.
b) Adult animals recovering from regression for another breeding season.
c) Animals in regression or quiescence and
d) Immature animals.

In plotting the diameter of the seminiferous tubules against the weight of the testes (Fig. 5) the first two groups have been segregated from the latter two in which spermatozoa are absent in the testes. The materials of groups (a) and (b) is arranged in ascending order of testes weight while that of groups (c) and (d) is arranged in descending order of testes weight. The maximum diameter of 190 micra is reached when the testes attain a weight of 3.5 grams and no further increase in tubule diameter takes place corresponding with further increase in testes weight to nearly 7.0 grams. It would therefore appear that increase in testes weight beyond 3.5 grams is due to increase in tubule

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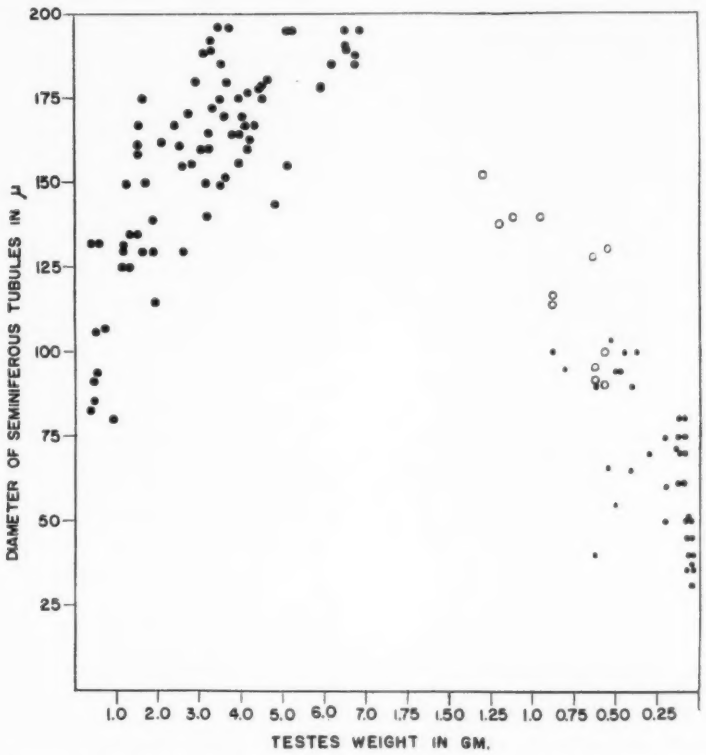

Fig. 5. Scatter diagram showing the relation of the diameter of the seminiferous tubules to the weight of the testes.

length than to tubule diameter. Similar observations where tubules elongate with increase in testes weight have been made by ALLANSON (1932, 1933), ROWLANDS and BRAMBELL (1934), DEANESLY (1935), HILL (1939) and PRASAD (1951). In the regressing testes the relationship approximates to a close fitting linear correlation. A reference to Fig. 4 showing the seasonal variation in the diameter of the seminiferous tubules shows the levels of regression of tubule diameter. The extent of regression of tubule diameter appears roughly to correspond with the different levels of development of the tubules at the height of activity. For example it is reasonable to assume that an individual with tubule diameter of 185 micra could not regress to the lowest of 85 micra but to about 130 micra. As the scatter diagram (Fig. 4) shows the levels of regression of seminiferous tubules noticed in a population consisting of several age groups in various stages of development, the increase in diameter of the tubules and their regression correspondingly vary.

Histology:— From the last week of April to the end of June the testes of adults are characterised by the presence of a thick fibrous tunica albuginea

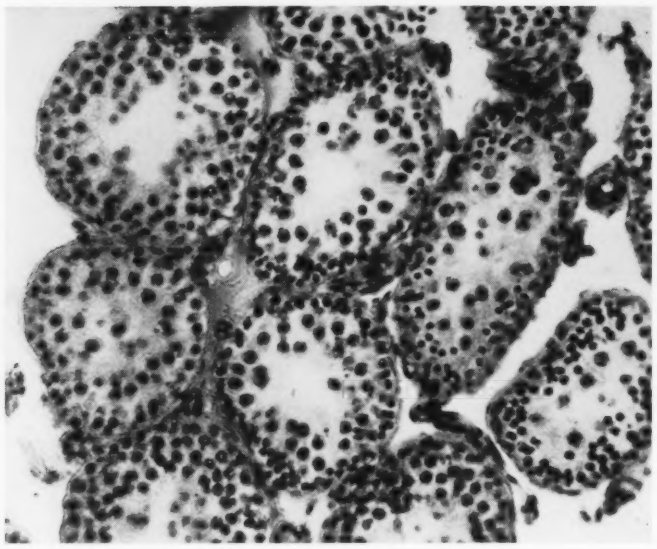

Fig. 6. Section of the testis of an adult gerbille in regression in May (Ta. 360). The lumina of the seminiferous tubules are empty and show the characteristic enlargement of the nucleus of the spermatogonial cells. X 210.

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presenting a crumpled appearance. They are relatively anaemic and the capillaries are sparse. The seminiferous tubules are small in diameter (about 90 micra), with large intercellular spaces between them. The majority of the spermatogonial cells are in a resting condition. A few mitoses are seen in the spermatogonial cells. It is interesting that at no period of the year is there a complete cessation of all activity in the testes so characteristic of hibernating species. The spermatogonia form a single layer surrounding the greatly reduced lumen. Sertoli nuclei are scattered among the spermatogonia. The regressed testes are also characterised by the presence of a few enlarged spermatogonial cells (Fig. 6), comparable with the similary enlarged "winter spermatogonia" described in the bat, mole and hedgehog by COURRIER (1923-quoted by ALLANSON 1934) and in the ferret by ALLANSON (1932). These enlarged cells are not common in the active testes. It is possible that the spermatogonia assume this enlarged condition in the resting state. By the end of March and April the seminiferous tubules of a number of animals show masses of degenerating spermatids and spermatocytes desquamated from the seminiferous epithelium into the lumen of the tubules. These degenerated masses are carried down the epididymis and vas deferens and are finally thrown out. This indicates the commencement of regression. Such degenerating masses are not noticed in the tubules of active testes.

About four to six weeks before the onset of reproductive activity in the

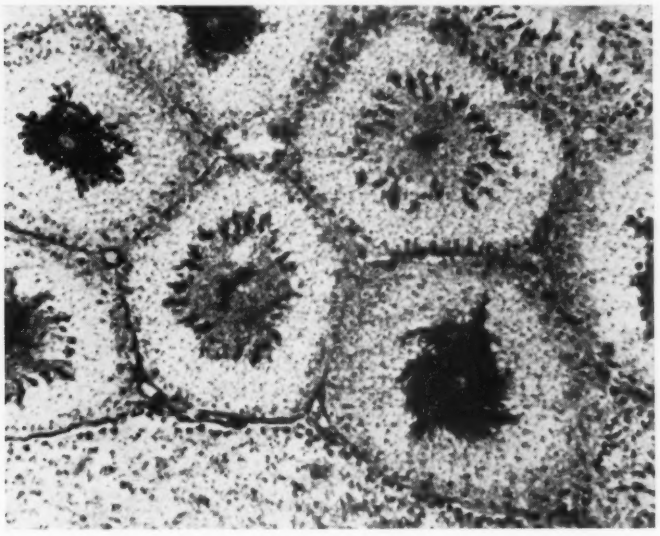

Fig. 7. Section of the testis of an adult in active state. X. 240 (Ta. 180 — October).

female (the activity in the female starts in the first week of September) the seminiferous tubules show signs of proliferation. From the beginning of July spermatogonial cells divide and the tubules contain spermatocytes arranged in two to three rows. Tubule diameter increases and they become more compact in arrangement and the intertubular spaces decrease considerably. The tunica albuginea thins out and shows an even surface. In the fully active testes the blood capillaries are distributed in larger numbers and the blood vessels stand out markedly over the transparent tunica albuginea in the form of well defined bands.

By the beginning of August all the males, those born in the latter part of the previous breeding season which were not yet mature, and those recovering from the quiescent period of regression in May and June show the transformation of spermatids into spermatozoa (Fig. 7). Active production of sperms continues unabated from August to the middle of March after which the rate slows down considerably. Counts of different cells in stages of spermatogenesis were made and the results will be described in detail in another paper.

Interstitial cells:— In animals exhibiting cyclic periodicity in breeding, the interstitial cells also show cyclic variation in size and presumably in activity. In view of the difficulty involved in the determination of the volume of the interstitial cells or their absolute number, the mean area of the cells was calculated and the seasonal variation of the area of the cells is shown in Fig. 8. The variation in the area of the interstitial cells roughly corresponds with the activity of the testes (cf. Figs. 3 and 4). In May and June when the testes

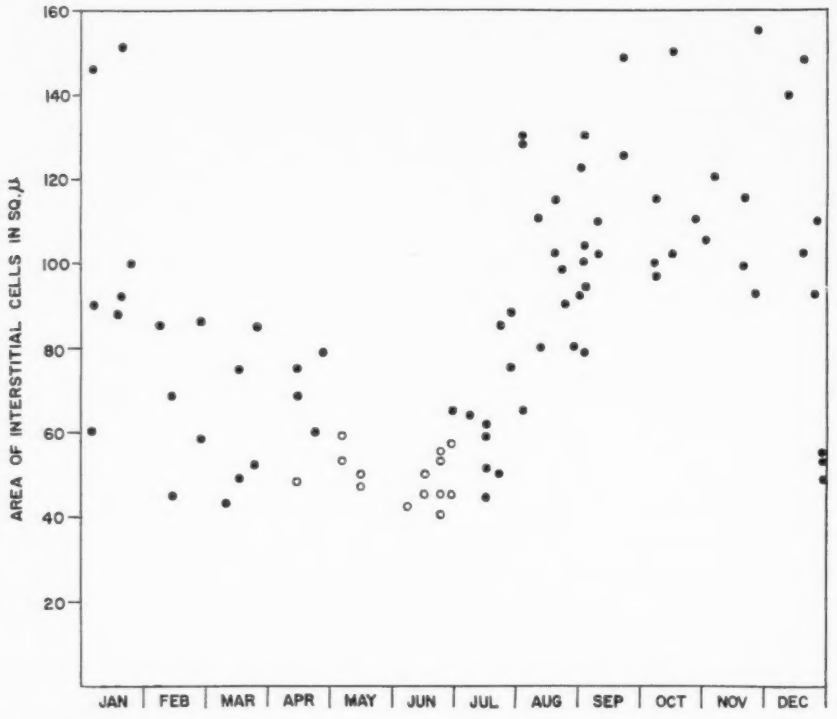

Fig. 8. Scatter diagram showing the seasonal variation in the area of the interstitial cells.

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are regressed the mean area of the interstitial cells varies from 45 micra to 65 micra while at the height of the breeding season it reaches about 155 micra. The variation in the size of the cells appears to vary directly with the curve of the weight of the testes and diameter of the seminiferous tubules in different months of the year.

The interstitial cells are small in the quiescent testes while in the active testes the cells are large and in many places are clustered together to form small 'nests'. These nests are commonly noticed in the active testes of a large number of other mammals also. There appears to be a relationship between the number of the interstitial cells and the intertubular connective tissue cells. In its active state (Fig. 9) the interstitial cell appears large with a large nucleus and a prominent nucleolus. At the commencement of regression of the testes the nucleus of the interstitial cells appears collapsed and the nucleolus becomes indistinct. The cell elongates and thins out (Fig. 10). With further elongation and reduction in the size of the nucleus which becomes oval and compact, the appearance of a typical connective tissue cell is reached. The reverse change is noticed at the commencement of the breeding season.

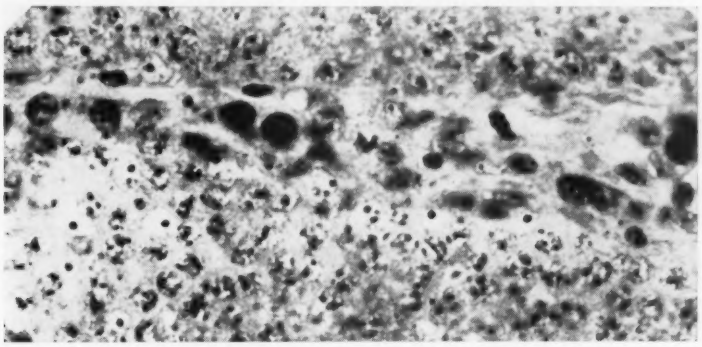

Fig. 9. Section of the testis of an adult in the active state to show the interstitial cells clustered together in large numbers. The interstitial cells are large with vesicular nuclei (Ta. 180 — October). X 590.

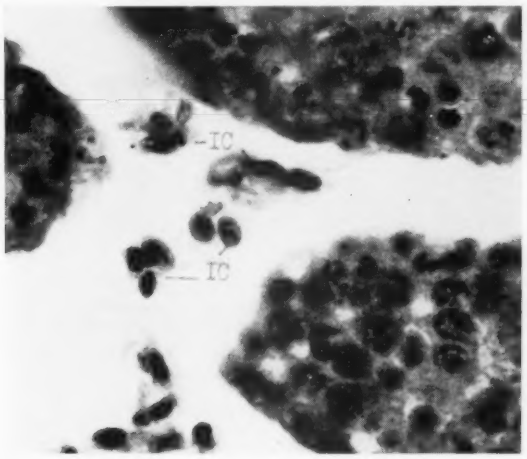

Fig. 10. Section of the testis of an adult gerbille in regression in May (Ta. 360). The interstitial cells are smaller in size and some are in various stages of elongation and conversion to connective tissue cells. X 590. IC-interstitial cells.

EPIDIDYMIS

The epididymis shows changes which appear to correspond with those of the testes. The weight of the epididymedes is plotted against the weight of the testes (Fig. 11) closely approximates to a linear correlation. The weight is lowest in May and June (0.110 grams) and rises gradually to a maximum of 2.34 grams in the breeding season. The reduction in epididymis weight is gradual and not so steep as in the testes at the end of the breeding season.

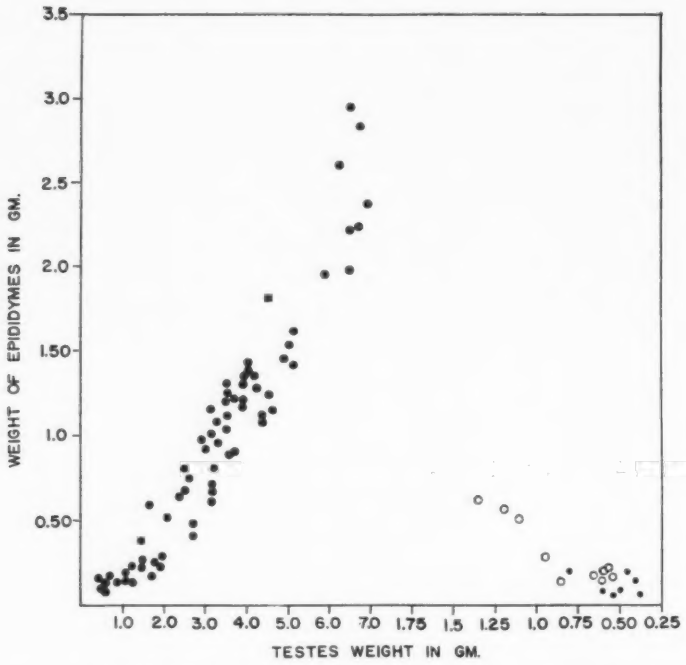

Fig. 11. Scatter diagram showing the relation of the weight of the epididymis to the weight of the testes.

This gradual fall in weight is probably due to the presence of desquamated spermatocytes and spermatids in the epididymal tubules long after the cessation of the production of sperms in the testes.

The diameter of the epididymal tubules at the body region is lowest in the quiescent period (115 micra) and reaches the maximum of 380 micra in the breeding season. During regression in May and June the decrease in tubule diameter is correlated with the absence of spermatozoa in the tubule and the thickening of the muscle layers around the tubules (Fig. 12). As the spermatozoa accumulate in the tubules at the commencement of the breeding season, tubule diameter increases and this is accompanied by the stretching of the muscle layers which decrease in thickness (Fig. 13).

If spermatozoa are present in the testes they also occur in the epididymis indicating that mature sperms can be found in the epididymal tubules almost immediately after their formation in the testis. With the advance of regression of the testis, cells desquamated from the seminiferous epithelium accumulate in the epididymal tubules and are eventually thrown out. A few show spermatozoa in the epididymal tubules when the testis is devoid of sperms indicating

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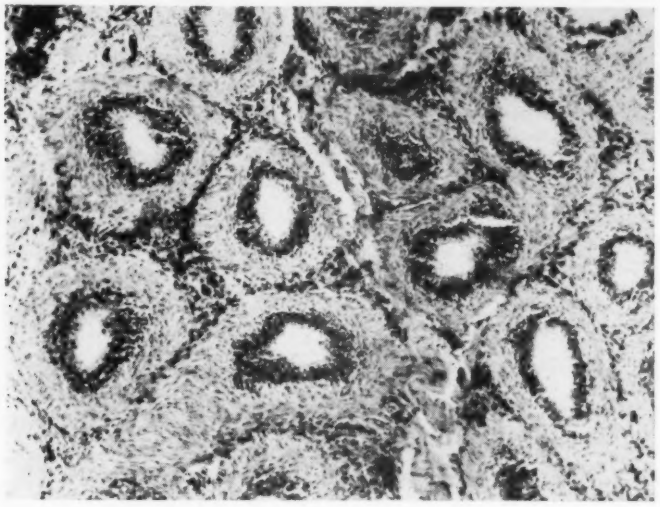

Fig. 12. Section of the mid-epididymis of an adult in regression in June (Ta. 383). The epididymal tubules are empty and the muscle layers are thick. X 100.

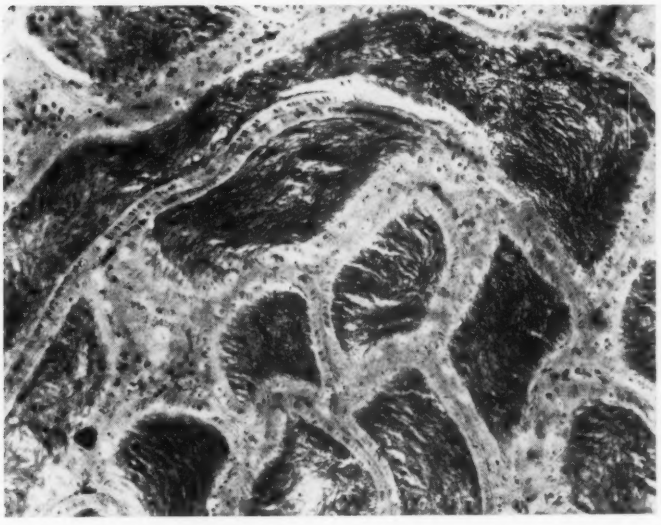

Fig. 13. Section of the mid-epididymis of an adult in the active state in September (Ta. 132). The epididymal tubules are gorged with spermatozoa. X 100.

Fig. 14. Section of the vas deferens of an adult in regression in May (Ta. 350). The musculature is thick. X 80.

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that sperms may persist in the epididymis for some time after complete cessation of activity in the seminiferous epithelium.

VAS DEFERENS

The diameter of the vas deferens varies with the cyclic reproductive activity of the animal. The diameter is lowest in the non-breeding season and increases with the onset of activity in the testes prior to the migration of sperms into the vas deferens. In the regressed state the vas is surrounded by thick circular and longitudinal muscle layers and the epithelium appears collapsed. Tubule diameter is lowest at this period and the lumen empty (Fig. 14). Changes occur in the tube prior to the migration of sperms and these are chiefly enlargement of the lumen, reduction in thickness of muscle layers, increase in the height of the epithelium and appearance of cilia, presumably stereocilia. At the time of regression in May and June the lumen may, for some time, be filled with desquamated cells from the testis.

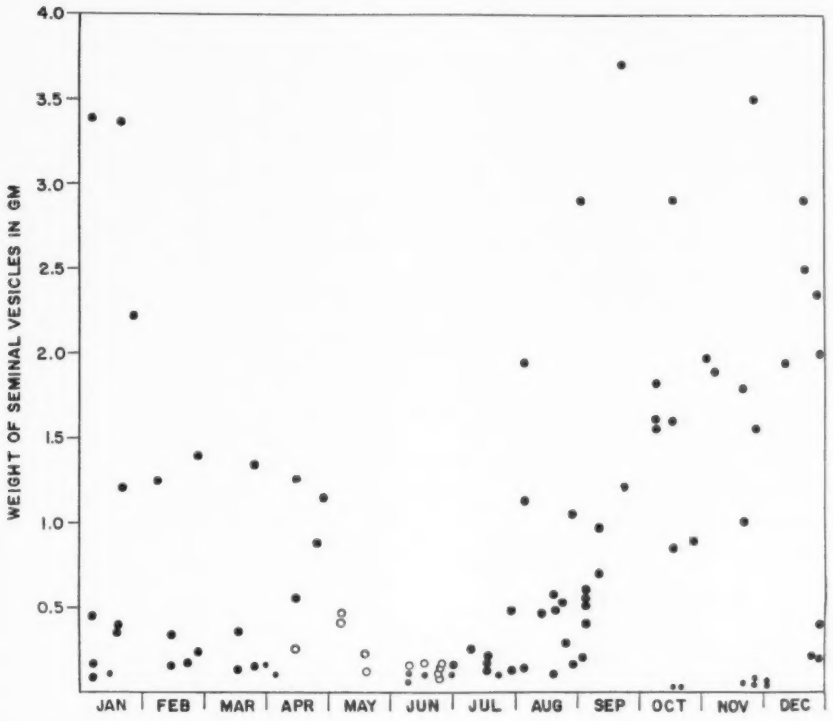

Fig. 15. Scatter diagram showing the seasonal variation in the weight of the seminal vesicles.

SEMINAL VESICLES

The seminal vesicles are the largest of the accessory glands of reproduction and reach a weight of over 3.75 grams at the height of the breeding season. During this period the tubules are greatly coiled and dilated with secretion. The weight of the glands of all animals plotted in Fig. 15 shows a steep fall in regression and a gradual increase there after commencing at the beginning of July and progressing to the completely active state in the breeding season. In the early stages of initiation of activity in July, it is possible to find a large number of animals with spermatozoa in the testis in which the seminal vesicles have not reached beyond 0.5 grams in weight. In males regressing for the second or subsequent season of their life, the tubules of the seminal vesiclees appear flabby and without any secretion during May and June, while this does not appear to be true either in immature animals or in those regressing for the first time. The size and gross appearance of the seminal vesicles would be easy guides for distinguishing the different groups of animals. During growth

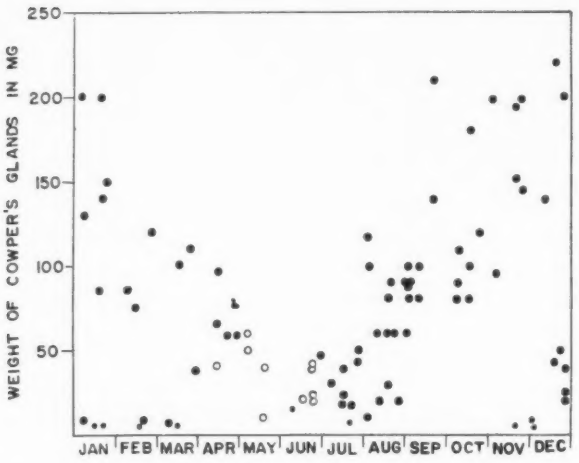

Fig. 16. Scatter diagram showing the seasonal variation in the weight of the Cowper's glands.

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and activity, the microscopic appearance of all types is the same. Since the histological variations of the accessory glands are too well known, reference is made here only to changes in the weight of the organ in the mature and immature animals. On account of the difficulty of separating the prostate glands no reference is made to changes in the weight of the gland, but it follows the same pattern as in the seminal vesicles.

COWPER'S GLANDS

The Cowper's glands vary from 5 to 225 milligrams in weight. The seasonal distribution of the weight of the glands (pair) shown in Fig. 16 closely follows the activity of the testes and other accessory organs of reproduction. The weight of the glands per pair is between 10 and 55 milligrams in the period of regression of May and June. In July and August there is a gradual growth and in some animals the weight has reached 225 milligrams by the end of November. In the immature animals the combined weight of the glands of the two sides is not above 15 milligrams in any season of the year.

PENIS

The seasonal changes in penis weight and size are relatively slight compared with other parts of the reproductive tract (Fig. 17). The weight of the penis in adults reaches a maximum of 600 milligrams. Heavy penis weights are recorded

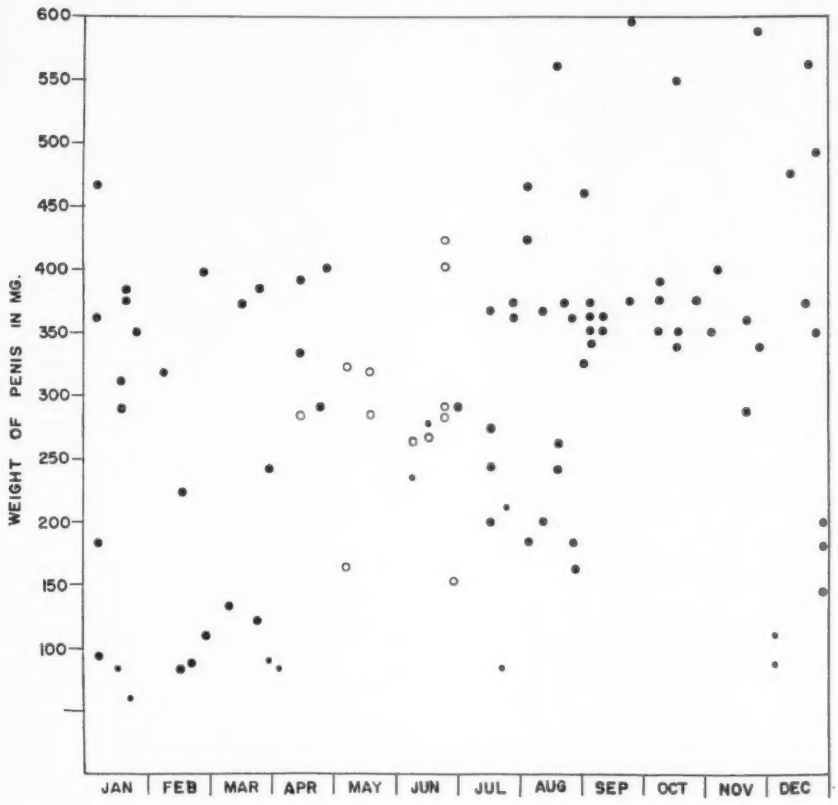

Fig. 17. Scatter diagram showing the seasonal variation in the weight of the penis.

in all months of the year indicating that the weight of this organ is more closely related to age of the animal rather than to periodicity in sexual activity. Hence, the weight of the penis could easily be used as one of the criteria for classifying the animals as immature or adults.

CORRELATION BETWEEN THE TESTES AND ACCESSORY ORGANS

The correlation diagrams (Figs. 18 and 19) clearly indicate the relation between the weight of the testes and the corresponding changes in the weight of the seminal vesicles and Cowper's glands. In the diagrams all animals with spermatozoa in the testes have been separated from immature animals and those in regression in which the production of spermatozoa has ceased completely. In all the groups the relation approximates to a linear correlation. The

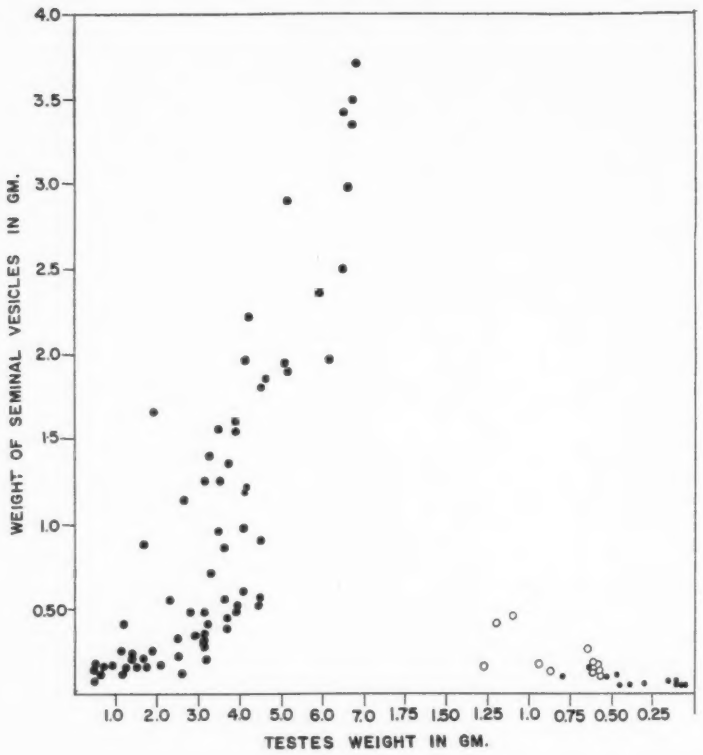

Fig. 18. Scatter diagram showing the relation between the weight of the Cowper's glands and the weight of the testes.

growth of the accessory organs shows an initial lag compared with the weight of the testes. Till the testes have reached a weight of 2.0 grams the accessory glands do not show any considerable increase in weight. From this stage onwards the rate of growth of the accessory glands is faster than that of the testes. There is a little spread of the weight of the seminal vesicles (Fig. 18) until after the testes reach 3.0 grams. This is probably due to the relatively slow growth of the vesicles in the younger animals.

DISCUSSION

Age of maturity:—

In the gerbille the earliest litters are brought forth in the second week of September (PRASAD 1954 a). The smallest animals with sperms in the testes were caught on the 31st December (Ta. 13, 16 and 25). These were first year

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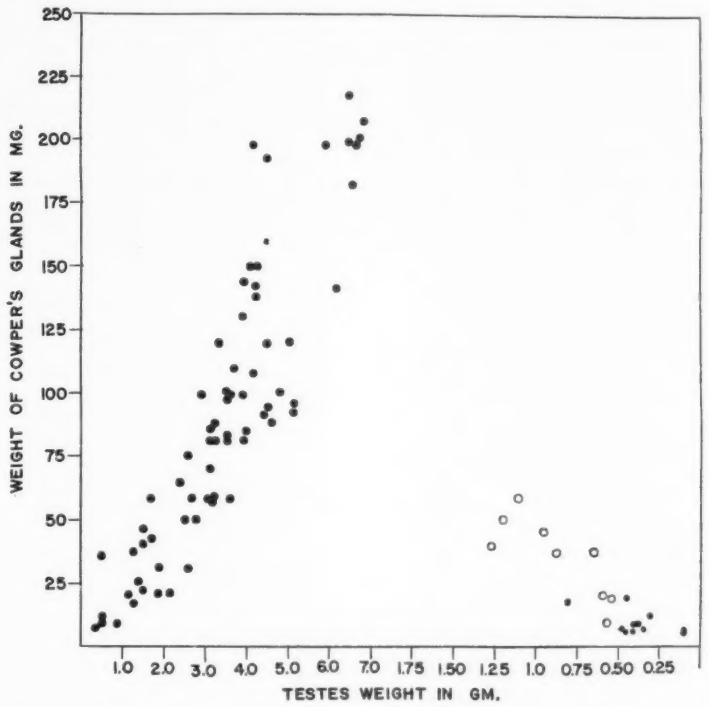

Fig. 19. Scatter diagram showing the relation between the weight of the seminal vesicles and the weight of the testes.

males from litters of the same breeding season. Hence, it is clear that the period of immaturity in the males is very short and that they become sexually mature in 12 to 14 weeks after birth. From December onwards successive waves of animals come to maturity dependent on the time of their birth. In litters which arrive at the end of the breeding season (February and early March), the growth of the testes and accessory organs of reproduction and the growth of the body do not synchronise. There is a definite lag in the development of the reproductive organs while the growth of the body proceeds at a steady pace as evidenced by the presence of animals with heavier body weight and yet with immature testes (Ta. 392, 396, 404, 428 and 432) in June and July. The lag exhibited by the reproductive organs of these immature animals continues through the period of regression for adults (May and June) till the activity is initiated in July at the commencement of the next breeding season.

Breeding season:—

Activity in the males as indicated by the presence of sperms in the testis and epididymis, extends from the commencement of July to the end of April. Sperms

are completely absent in the testes and epididymis in May and June. The period of regression or quiescence is therefore, very short in the male gerbille, while in the female quiescence or anestrum extends over a longer period, from March to early September. At no time of the year is there a complete cessation of all activity in the testes. The changes in the testes and accessory organs of reproduction indicate gradual growth in preparation for the breeding season after which the component parts of the reproductive system, except the penis, regress into the inactive state. Sexual activity in the male commences a few weeks earlier than in the female and continues a few weeks after the cessation of reproduction in the female.

Interstitial cells:—

The interstitial cells of the testes have formed the subject of discussion in regard to (1) the relation of their number and size to spermatogenic activity (2) their origin in animals with cyclic reproductive activity and (3) their function.

In animals with a regular periodicity in reproductive activity, there is an intimate relationship between the absolute number of the interstitial cells and the activity of the seminiferous epithelium. In such animals it has been shown by REGAUAD (1904) and TANDLER and GROSZ (1911) in the mole, RASMUSSEN (1917) in *Marmota monax,* BISSONETTE (1930) in the British starling, MILLER (1939) in *Myotis lucifugus* and *Myotis griscscens* and SESHACHAR (1941) in the apodan *Ichthyophis,* that the interstitial cells are greatest in number, largest in size and most active in the regressing testes and least in number, smallest in size and inactive at the height of the breeding season and spermiogenic activity.

On the other hand, it has been shown by GROOM (1939) in *Apodemus* (two species) and *Pteropus,* by DEANESLY (1935) in the stoat, by HILL (1939) in the weasel and by GOPALAKRISHNA (1949) in *Scotophilus,* that there is an absolute increase in the number and size of the interstitial cells correlated with increased spermiogenic activity and that they are reduced both in size and activity in the non-breeding period.

In the gerbille, *Tatera,* the size of the interstitial cells is least in the quiescent period of May and June and it increases gradually to its maximum size in the breeding season. The increase and decrease in the size of the interstitial cells closely parallel the increase and decrease in testis activity.

The periodic increase in the number of interstitial cells in animals with cyclic sexual activity may be either due to the increase in number of the existing interstitial cells by cell division or by transformation of intertubular and stromal cells into interstitial cells. Both amitotic and mitotic divisions of the interstitial cell nuclei have been reported by different workers. RASMUSSEN (1917) observing clusters of interstitial cells and some bi-nucleate and multi-nucleate cells in *Marmota monax,* concluded that they represent products of division of a

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parent cell. He did not observe any interstitial cells in division. In the absence of division, it has been suggested by ALLEN (1904) working on the rabbit, WHITEHEAD (1904) on pig, KINGSBURY (1914) on the cat, BISSONETTE and CHAPNICK (1930) on the European starling, BASCOM (1923) on cattle, HOOKER (1930) on the bull and GOPALAKRISHNA (1949) on *Scotophilus* that the intertubular connective tissue cells and stromal cells transform themselves into interstitial cells during the period preparatory to the breeding season. This theory means that in the period when the interstitial cells are largest in number the stromal cells and intertubular connective tissue cells are least and when the interstitial cells decrease, the connective tissue and stromal cells increase.

In *Tatera*, clusters of interstitial cells are found in the active testis which correspond in appearance to 'nests' described in the testis of other mammals. This grouping appears to signify nothing more than a mere approximation of cells, in view of the fact that in the gerbille none of the interstitial cells have been seen in division. On the other hand, different stages in the transformation of intertubular connective tissue cells into interstitial cells are often observed. Though an inverse numerical correlation between the two types of cells could not be established, it seems probable that they are interconvertible.

The interstitial cells are considered to be the main site of production of testis hormone or androgen. The evidence regarding the secretory nature of the interstitial cells is purely circumstantial and is evidenced by the concomittant increase in interstitial size and by the exaggeration of secondary sexual characters and sexual behaviour, the latter two known to result from increased production of the male hormone. HOOKER (1944) states that the manifestations of secondary sex characters at puberty are not caused by changes in number and activity of the interstitial cells. The male testicular hormone, according to him, is not solely responsible for changes occurring at puberty, since he did not find any change in the androgen content of the bull testis at puberty. The function of the interstitial cells becomes very difficult to assess in animals with cyclic reproductive activity, in which periodic increase and decrease of these cells also occurs. In some animals these cells are larger and more numerous in the quiescent period while in the others the reverse is true. They behave differently in different animals. In *Pteropus geddei* it has been shown by GROOM (1940) that a curious correlation exists between the association of the males and females and abundance of the interstitial cells in the testes. After copulation the males and females separate and live in different colonies. When the interstitial cells are most reduced the males begin to live in isolation. He therefore concludes that the male hormone may, to some extent, control the purely social behaviour of the bats.

ENVIRONMENTAL FACTORS AFFECTING THE BREEDING SEASON

With the onset of maturity, seasonal sexual activity is evident in most species. This is obvious in animals living in the arctic and sub-arctic regions while it is less marked in those of the tropical and sub-tropical regions.

In order to obtain a clear idea of the environmental factors in relation to sexual periodicity of the gerbille, it is necessary to summarise briefly the climatic changes in Bangalore. Bangalore lies in the middle of the Deccan plateau at 77° 38′ east longitude and 12° 58′ north latitude at a height of 3050 feet above the mean sea level. The climatic changes are summarised in the following table, Table II.

TABLE II.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature Maximum	80	86	90	91	93	84	81	81	82	82	79	78
Temperature Minimum	57	60	65	69	69	67	66	66	65	65	62	58
Rainfall in inches	0.2	0.3	0.4	1.6	4.2	2.7	3.9	5.0	6.7	5.9	2.7	0.4
Rainy days	0.5	0.4	0.8	2.6	6.6	5.9	8.0	9.3	8.9	8.5	4.5	1.2
Day length in hours	11.28	11.40	12.05	12.25	12.55	13.00	12.50	12.35	12.11	11.50	11.30	11.20
Night length in hours	12.32	12.20	11.55	11.35	11.05	11.00	11.10	11.25	11.49	12.10	12.30	12.40

A number of environmental factors appear to affect the sexual cycles in vertebrates, chief among them being:—

(1) Light, day length and night length, (2) temperature (3) food and (4) rainfall. No single factor is itself solely responsible for inducing sexual periodicity of breeding.

Light:—

It has generally been assumed that animals with a summer breeding season can be induced to breed at other times of the year by increasing the length of day by artificial means. Positive results on the above lines were obtained by ROWAN (1929) in the junco and BISSONETTE (1930) in the starling. BAKER and RANSOM (1932) induced the field mouse *Microtus agrestis* to cease reproduction by reducing the day length from 15 to 9 hours. Females respond to modification of light ration while the males remained potent and fertile in spite of changes in the duration of day length, indicating that other factors beside light were concerned with sexual cycles in males. Similar results were obtained by

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BISSONETTE (1932) on the ferret. On the other hand, MARSHALL and BOWDEN (1934) showed that in ferrets, if estrus has once set in at the normal breeding season of the year, reduction of light fails to hold it back.

In tropical and sub-tropical regions, climatic conditions are so uniform throughout that conditions are conducive for animals to breed continuously. Even under such conditions most terrestrial animals show seasonal changes in reproductive activity. BAKER (1947) observes that the insectivorous bat *Miniopterus australis* inhabiting dark caves in the northern New Hebrides, exhibits a clearly marked breeding season in the first few days of September only. Commenting on the limitation of light as an environmental factor in controlling sexual periodicity BAKER (1947—page 254) remarked "No animal living in a strongly seasonal climate could have a more sharply defined breeding season. This seems all the more extraordinary when it is remembered that this little bat (*Miniopterus australis*) hangs throughout the period of day light, till about ten minutes before sunset, in a dark and almost thermostatic cave".

The gerbille *Tatera,* is nocturnal and is confined to its burrows during the day. The animal leaves its burrows at dusk and returns to its hideout at dawn. Hence, it is almost impossible that light of day has any significant role to play in the seasonal sexual activity of the gerbille.

Day length and night length:—

The day length is longest in May and June and gradually decreases to the lowest limit in November and December. Consequently the activity of the gerbilles in the fields is greatest in the period of long nights and the activity is least as the length of night decreases with the approach of the hot weather period of April to June. The longest night is one hour and forty minutes longer than the shortest. ROWAN (1932) found that it was possible to induce winter development of the testes of juncos by compulsory excercises forced on birds kept in complete darkness, the intensity of light being below the point at which light begins to be effective by itself. In the gerbille, the cyclical variation of night length and correlated physical activity correspond with the sexual activity of the male. The female gerbilles appear to react more sharply to changes in the length of night than the males. A reference to Fig. 20 shows that the breeding in the females commences in September when the length of night is 11 hours and fortynine minutes and when the light length is reduced to the lower limit of 11 hours and fifty minutes in March, reproductive activity ceases completely.

Temperature:—

In temperate regions many mammals hibernate during the winter months when external temperature is lowest. With the onset of spring and gradual

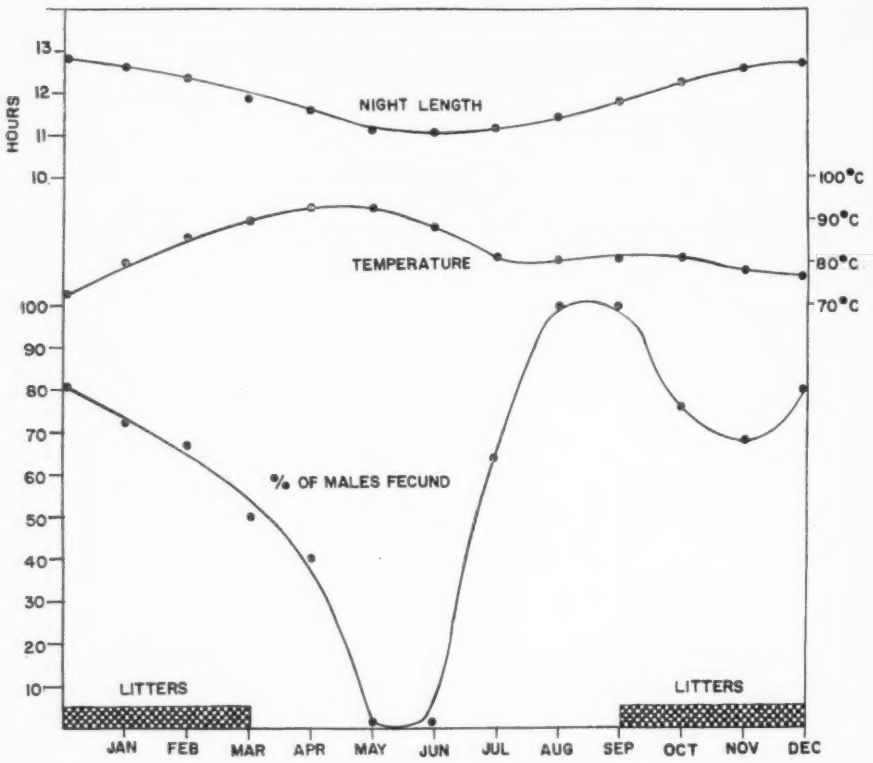

Fig. 20. Composite graph showing the relation of environmental changes with the seasonal sexual activity of the gerbille.

The curve at the top shows the variation in the length of night in hours.

The curve in the middle indicates the variation in air temperature by day in degrees centigrade.

The curve at the bottom shows the monthly percentage of males with spermatozoa in the testes.

The shaded portion at the bottom line shows the extent of the breeding season in the female when litters are born.

rise of temperature, the animals come out of dormancy and become sexually active.

In sub-tropical countries the mammals do not hibernate, since the temperature is maintained at a fairly high level throughout the year. The gerbilles remain during the day in deep dark ground burrows, where temperature is almost uniform throughout. They are active in the fields during the night when temperature variation is not very great in the different seasons of the year. The extreme limit of variation in day or night temperature (air temperature) is not more than 15° in any season of the year. The composite graph Fig. 20

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shows that there is an apparent correlation between the variation of day temperature and sexual periodicity of the male gerbille. Why it is so is not clear.

Food:—

Another variable factor related to seasonal breeding is the type and quantity of food available at different times of the year. Dealing with a number of essential food factors causing sterility in mammals, CUNNINGHAM and HOPKIRK (1935) observed that "full reproductive capacity is reached only when the nutritive environment is adequate and complete while sterility may be caused by the restriction of food intake either in a qualitative or quantitative manner". It has been shown experimentally that food deficiencies condition alterations in reproductive cycles. RICHTER, HOLT and BARELARE Jr. 1938) using the self-selection method to study the nutritional requirement of food for rats for growth and reproduction showed that rats provided with a variety of food stuffs make a systematic selection and maintain themselves at a comparatively high nutritional level and pass through regular sexual cycles. Vitamin E deficiency causes a retardation of the maturation of the male germ cells. Commenting on the importance of food in relation to the breeding seasons of *Peromyscus maniculatus* and *P. boylei* JAMESON Jr. (1953) observes that abundance and scarcity of food parallel the autumnal period of sexual activity and quiescence and believes that food is a basic determinant of autumnal reproduction in these mice.

A quantitative analysis of the food of the gerbille (PRASAD, 1954 b) shows that there is a seasonal variation in the items of food eaten and these are mainly dependant on the constancy of crops grown in the fields. The main items of food eaten are seeds of various food crops, rhizomes of a variety of grasses and insects. With the onset of the south-west monsoon in the last week of May, farming activity is initiated and seeds are sown by the last week of June. From this period to the end of the harvest season the fields are full of either seeds or seedlings and a variety of insects that accompany them as pests. Harvesting of all crops is completed at the beginning of February. Scattered seeds are available in the fields till the end of March or the beginning of April after which the gerbilles depend mostly on the rhizomes of grasses and other miscellaneous items of food. The period from April to the middle of June is the leanest of the year when the gerbilles become omnivorous. Cannibalistic remains are common in the stomachs of gerbilles during October to January when the younger animals leave their parent burrow to establish new homes.

The reproductive activity of the male gerbille extends from the beginning of July to the last week of April of the following year and it corresponds with

the period of availability of an abundant supply of food in the environment. The period of regression in May and June is one when food is scarce. Sexual activity is initiated with the inititation of farming and sowing of seeds in the last week of June or early July. The young are born from the middle of September, at a time when the surrounding fields are full of seedlings and seeds and various other items of food and the breeding season in the female and production of litters ceases in March after which scarcity of food occurs. It is definitely disadvantageous for the young to be born during this period. Though a chemical analysis of the food items consumed in the different seasons of the year has not been made, it seems probable that availability of food in adequate quantities is one of the important factors determining the periodicity of sexual activity in the gerbille.

Rainfall:—

Rainfall is an environmental factor of great importance to animals of the tropical and sub-tropical regions where there are regular alternations between the wet and dry seasons. Aquatic animals react more sharply to rain and may become sexually mature and spawn after the onset of rains. BAKER (1938) cites a number of examples of lizards and birds of Ceylon and India which correlate their reproductive periods with the commencement of rains. It has been suggested by BAKER (1938) that the "receptor whose stimulation causes certain animals to breed is unknown, nor is it clear whether the stimulant is the rain itself or the small saturation deficit of the atmosphere or the existence of ponds or floods. Green vegetation resulting from rain, rather than rain itself causes most birds to breed and actual drought may act by reducing the food supply".

In Bangalore the south-west monsoon commences in the last week of May and rainfall continues till the end of November. Reproductive activity of the male gerbilles is initiated at the commencement of July when the sowing operations would have been completed after the onset of the first showers in May and June. It it possible that rain could, at best, be an indirect factor in the sense that the onset of rains is accompanied by increase in food supply and the development of green vegetation. The possible role of increased food supply in the regulation of reproductive cycle of the male gerbille has already been discussed.

Problem of internal rhythm:—

In the absence of a decisive role of the environment in the regulation of breeding, it has been suggested that in each animal there exists an internal physiological or reproductive rhythm, with an approximate periodicity of one year. That such a rhythm exists has been shown by MARSHALL and BOWDEN

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(1932) in the ferret where reduction of light and imposition of darkness did not prevent the maturation of the gonads once estrus set in at the normal breeding season of the year. In migratory birds crossing the hemispheres it has been shown by BAKER and RANSOM (1938) that "the internal rhythm is some times so strong as to cause a southern hemisphere bird to breed at the locally 'wrong' time of the year when introduced into the northern hemisphere. Internal rhythm may often play an important part in determining the onset of breeding seasons a considerable time before the other proximate causes stimulating reproduction are beginning to be effective". BULLOUGH (1951) puts forward a theory that "some if not all vertebrates, possess an internal rhythm which is more or less of a vague kind and which in normal circumstances is rendered more precise in its time of action by the effect of the seasonal variation of environment".

In the gerbille, various environmental factors that have been considered exert little or no influence on the regulation of sexual activity. It is therefore reasonable to suggest that there exists a similar internal rhythm or reproductive rhythm which induces seasonal sexual activity in the male gerbille, conditioned slightly by changes of environment, chief among which is an adequate supply of food.

SUMMARY

The reproductive cycle of the male gerbille *Tatera indica cuvierii* (WATERHOUSE) has been studied by observations on 122 males collected from the fields near Bangalore (Mysore State-India), over a period of two years.

Litters are born from the last week of September to the first week of March the following year. The period of immaturity in the males is only 12 to 14 weeks.

The males are sexually active from July to the end of April and the reproductive organs are regressed in May and June. The breeding season recurs annually at about the same time. At no time of the year is there cessation of all activity in the seminiferous epithelium in adults. The males become active a few weeks before the onset of activity in the female and remain sexually active for a few weeks after the cessation of breeding in the female.

The testes and accessory organs of reproduction undergo considerable reduction in size and weight during the period of regression.

There is a cyclic change in the appearance of the interstitial cells. They are largest in the active testes and are greatly reduced in the regressed testes. The increase in their number in the active testes is probably due to the transformation of intertubular connective tissue cells and stromal cells into interstitial cells.

Various environmental factors like light and length of day and night, tem-

perature, food and rainfall are considered in relation to the sexual periodicity in reproductive activity. It would appear that in this species light, temperature and rainfall do not have any direct bearing on the regulation of reproductive cycle. With the increase in the length of night there is greater sexual activity in the gerbilles. Increased physical activity during the period of long nights may have some effect on the conditioning of the physiology of the reproductive system.

A rough quantitative analysis of the food of the gerbille shows that there is a seasonal variation in the food consumed and there exists a correspondance between the period of reproductive activity and the availability of food in sufficient quantities which plays a great part in determining sexual periodicity.

In the absence of any environmental factor playing a decisive role in the regulation of sexual cycles, it is suggested that an internal rhythm is operative with a periodicity of one year responsible for seasonal sexual activity in the male gerbille.

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ACKNOWLEDGEMENT

My sincere thanks are due to Dr. B. R. SESHACHAR and Dr. L. S. RAMASWAMI for their guidance and encouragement during the progress of this work in the Department of Zoology, Central College Bangalore, India and to Dr. H. W. MOSSMAN for criticism and valuable suggestions.

APPENDIX I.

Index Number	Date	Body weight	TESTES				EPIDIDYMIS			Seminal vesicle weight-grams	Cowper's gands weight-grams	Penis weight grams
			Weight-grams	Seminiferous tubule diameter. Micra.	Area of Interstitial cell square Micra.	SPERMS	Weight-grams	SPERMS	Vas deferens. sperms			
January												
250	5	70	0.100	80	—	—	—	—	—	—	—	—
267	28	45	0.120	75	—	—	—	—	—	—	—	—
264	21	80	0.460	85	—	—	—	—	—	—	0.004	0.060
252	15	70	0.470	94	—	—	—	—	—	0.100	0.005	0.080
242	5	92	0.507	100	—	+	—	+	+	0.060	0.007	0.095
241	5	110	1.900	130	60	+	0.220	+	+	0.160	—	0.190
258	18	120	3.170	160	—	+	1.160	+	+	0.352	0.086	0.290
259	18	150	3.500	150	87	+	1.200	+	+	0.370	—	0.310
243	5	173	3.950	155	90	+	1.300	+	+	1.480	0.130	0.360
261	20	180	4.130	160	92	+	1.350	+	+	1.200	0.140	0.380

APPENDIX I—continued

266	25	175	4.200	162	100	+	1.280	+	+	2.220	0.150	0.350
240	5	209	6.500	190	146	+	2.960	+	+	3.440	0.199	0.465
262	20	210	6.72	185	151	+	2.80	+	+	3.350	0.200	0.380
February												
43	16	58	0.042	45	—	—	—	—	—	—	0.004	—
41	14	80	0.050	50	—	—	—	—	—	—	—	—
57	27	92	0.460	82	—	+	—	—	—	—	—	—
38	14	96	0.484	85	45	+	—	+	+	0.140	—	—
292	22	115	0.507	132	—	+	0.089	+	+	0.160	0.007	0.085
55	27	125	1.120	125	58	+	0.180	+	+	0.240	—	0.110
39	14	135	2.560	129	68	+	0.700	+	+	0.320	0.075	0.220
289	8	145	3.136	140	85	+	1.100	+	+	1.250	0.086	0.315
53	27	165	3.260	193	85	+	1.050	+	+	1.400	0.120	0.399
March												
312	16	96	0.036	35	—	—	—	—	—	—	—	—
315	16	60	0.040	38	—	—	—	—	—	—	—	—
303	8	90	0.063	50	—	—	—	—	—	—	—	—
321	23	60	0.066	50	—	—	—	—	—	—	—	—
65	10	50	0.090	70	—	—	—	—	—	—	—	—
60	10	80	0.098	75	—	—	—	—	—	—	—	—
63	10	95	0.477	131	43	+	0.070	+	+	—	0.004	0.130
70	31	100	0.492	95	—	+	0.100	+	+	0.130	—	—
307	16	115	0.610	95	49	+	0.120	+	+	—	—	—
69	31	72	0.630	40	—	—	—	—	—	—	—	—
320	23	105	0.690	106	52	+	0.150	+	+	0.160	—	0.120
306	16	120	2.960	179	75	+	0.980	+	+	0.350	0.100	0.370
322	23	150	3.692	196	85	+	1.236	+	+	1.350	0.110	0.380
April												
340	23	80	0.060	50	—	—	—	—	—	—	—	—
339	23	100	0.090	55	—	—	—	—	—	—	—	—
348	29	85	0.100	70	—	—	—	—	—	—	—	—
328	4	105	0.540	103	—	—	0.050	—	—	0.100	—	0.080
332	14	130	0.640	127	48	—	0.160	—	—	0.250	0.039	0.280
343	23	120	1.659	175	60	+	0.580	+	+	0.880	0.060	0.290
334	14	130	2.350	168	68	+	0.630	+	+	0.541	0.066	0.330
347	29	135	2.642	169	79	+	0.750	+	+	1.160	0.060	0.400
336	14	160	3.516	186	75	+	1.250	+	+	1.258	0.098	0.390
May												
363	17	95	0.031	30	—	—	—	—	—	—	—	—
357	17	91	0.035	35	—	—	—	—	—	—	—	—
362	17	120	0.059	45	—	—	—	—	—	—	—	—
361	17	140	0.581	84	50	—	—	—	—	—	—	—
360	17	150	0.942	139	47	—	0.260	—	—	0.143	0.040	0.316
353	6	150	1.100	140	53	—	0.500	—	—	0.446	0.060	0.320
350	6	120	1.200	137	59	—	0.540	—	—	0.400	0.050	0.160

APPENDIX I—continued

June												
399	16	90	0.199	74	—	—	—	—	—	—	—	—
377	9	105	0.296	71	—	—	—	—	—	0.050	0.014	0.230
396	16	110	0.400	90	—	—	—	—	—	—	—	—
392	16	110	0.440	100	—	—	0.190	—	—	0.100	0.020	0.260
395	16	110	0.500	55	—	—	—	—	—	—	—	—
404	30	105	0.560	66	—	—	—	—	—	—	—	—
406	30	137	0.570	92	45	—	—	—	—	0.098	0.020	—
376	9	120	0.587	100	42	—	0.210	—	—	0.150	—	0.260
380	9	100	0.590	90	—	—	0.080	—	—	0.120	—	—
393	16	125	0.590	95	50	—	0.110	—	—	0.160	—	0.250
382	24	140	0.599	92	45	—	0.190	—	—	0.090	0.020	0.294
383	24	150	0.860	114	53	—	0.124	—	—	0.110	0.038	0.420
407	30	130	0.880	118	56	—	0.120	—	—	—	—	0.150
384	24	130	1.260	152	55	—	0.600	—	—	0.140	0.047	0.400
405	30	137	1.540	159	65	+	0.180	—	—	0.153	0.047	0.290
July												
428	22	100	0.386	100	—	—	0.049	—	—	—	—	0.080
423	16	120	0.450	92	44	—	0.130	—	—	—	—	0.245
432	22	100	0.800	95	50	+	0.140	+	+	0.100	0.007	0.210
433	22	105	1.120	130	84	+	0.190	+	+	—	0.018	—
424	16	130	1.230	150	51	+	0.220	+	+	0.120	0.039	0.365
422	16	135	1.270	132	59	+	0.160	+	+	0.160	0.018	0.270
442	28	150	1.460	162	88	+	0.230	+	+	0.120	0.039	0.360
420	16	110	1.450	137	62	+	0.235	+	+	0.200	0.022	0.200
411	7	145	1.850	140	64	+	0.250	+	+	0.250	0.030	—
438	28	155	2.780	165	74	+	0.490	+	+	0.490	0.050	0.360
August												
90	3	100	0.950	80	65	+	0.120	+	+	0.150	0.010	0.180
458	28	120	1.720	150	—	+	0.180	+	+	0.150	0.020	0.160
95	11	102	1.970	116	80	+	0.266	+	+	—	0.020	0.200
456	28	130	2.060	166	81	+	0.510	+	+	0.160	0.020	0.180
101	19	125	2.580	155	—	+	0.430	+	+	0.100	0.029	0.260
454	19	140	3.120	189	102	+	0.660	+	+	0.490	0.060	0.235
114	25	130	3.160	150	90	+	0.700	+	+	0.280	0.060	0.360
97	11	138	3.600	151	110	+	0.870	+	+	0.479	0.060	0.365
452	19	130	3.620	180	115	+	0.880	+	+	0.560	0.080	0.560
109	22	150	4.440	177	98	+	1.080	+	+	0.520	0.090	0.370
87	3	160	4.800	146	128	+	1.450	+	+	1.910	0.120	0.449
86	3	152	5.030	155	130	+	1.521	+	+	1.100	0.100	0.420
September												
137	3	110	1.540	166	79	+	0.398	+	+	—	—	—
139	4	125	3.200	165	94	+	0.800	+	+	0.400	0.080	0.340
125	1	165	3.200	190	92	+	0.590	+	+	0.200	0.060	0.320
141	10	123	3.300	173	102	+	0.940	+	+	0.730	0.080	0.350

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147	10	120	3.500	196	110	+	1.040	+	+	0.980	0.099	0.360
138	3	140	3.950	175	104	+	1.360	+	+	0.500	0.100	0.350
134	3	140	4.000	170	100	+	1.350	+	+	0.600	0.085	0.360
149	21	178	4.140	169	125	+	1.379	+	+	1.200	0.140	0.370
132	3	150	4.450	178	130	+	1.100	+	+	0.550	0.090	0.370
126	1	179	5.100	195	122	+	1.439	+	+	2.910	0.090	0.460
150	21	205	6.890	194	148	+	2.340	+	+	3.700	0.210	0.600
October												
179	20	15	0.050	40	—	—	—	—	—	—	—	—
180	20	23	0.060	45	—	—	—	—	—	—	—	—
174	16	29	0.085	50	—	—	—	—	—	—	—	—
163	7	150	3.500	175	100	+	1.100	+	+	1.540	0.080	0.370
175	16	165	3.590	170	102	+	1.800	+	+	0.850	0.120	0.370
177	16	140	3.950	165	—	+	1.220	+	+	1.600	0.080	0.340
166	8	178	4.130	176	97	+	1.370	+	+	1.600	0.110	0.390
180	27	178	4.500	175	110	+	1.800	+	+	0.900	0.120	0.370
164	7	160	4.600	180	115	+	1.150	+	+	1.850	0.090	0.350
178	16	200	6.590	190	150	+	2.190	+	+	2.990	0.180	0.370
November												
220	26	36	0.098	60	—	—	—	—	—	—	—	—
204	20	35	0.100	75	—	—	—	—	—	—	—	—
218	26	400	0.160	80	—	—	—	—	—	—	—	—
215	26	150	3.980	165	93	+	1.260	+	+	1.560	0.145	0.340
202	20	160	4.090	160	99	+	1.400	+	+	0.990	0.150	0.290
181	2	150	4.110	168	105	+	1.330	+	+	1.980	0.199	0.350
200	20	170	4.500	175	115	+	1.250	+	+	1.800	0.195	0.360
192	6	192	5.160	195	120	+	1.600	+	+	1.900	0.095	0.400
211	26	205	6.726	189	155	+	2.220	+	+	3.500	0.199	0.340
December												
7	3	34	0.100	50	—	—	—	—	—	—	—	—
1	3	85	0.390	65	—	—	—	—	—	—	—	—
13	30	90	1.160	90	49	+	0.159	+	+	0.400	0.020	0.145
16	30	92	1.398	133	53	+	0.185	+	+	0.190	0.025	0.200
25	31	97	1.719	130	55	+	0.200	+	+	0.200	0.040	0.180
237	24	135	2.550	160	92	+	0.800	+	+	0.220	0.050	0.350
26	19	141	3.080	160	102	+	0.920	+	+	0.290	0.070	0.370
239	26	190	5.980	178	110	+	1.950	+	+	2.350	0.200	0.495
233	11	185	6.209	185	140	+	2.620	+	+	1.960	0.140	0.480
236	20	200	6.500	196	148	+	1.990	+	+	2.500	0.220	0.560

In each month the data are arranged in order of increasing testes weight.

LITERATURE CITED

ALLANSON, MARJORIE, 1932. The reproductive processes of certain mammals. Part. III. The reproductive process in the male ferret. Proc. Roy. Soc. Lond, *110.*

— 1933. The reproductive process of certain mammals. Part. IV. Changes in the reproductive organs of the male grey squirrel. *Sciurus carolinensis.* Phil. Trans. Roy. Soc. Lond. Ser. B. *222.* 79.

— 1934. The reproductive processes of certain mammals. Part VII. Seasonal variation in the reproductive organs of the male hedgehog. Phil. Trans. Roy. Soc. Lond. Ser. B. *223.* 277.

ALLEN, B. M., 1904. The embryonic development of the ovary and testis of mammals. Amer. J. Anat. B. *3.*

BAKER, J. R., 1938. The evolution of the breeding seasons. Evolution-Essays in aspects of evolutionary biology. Oxford. p. 161.

— 1947. The seasons in a tropical rain forest — part VI. Lizards (Emoia). J. Linn. Soc. Zool. Soc. Lond. *41.* 243.

— 1947. The seasons in a tropical rain forest — part VII. Final report and summary. Ibid. *41.* 248.

BAKER, J. R. and RANSOM, R. M., 1932. Factors affecting the breeding seasons of the field mouse *Microtus agrestis.* Part. I. Light. Proc. Roy. Soc. Lond. *110.* 313.

BAKER, J. R. and HARRISON, T. H., 1936. The seasons in a tropical rain forest (New Hebrides) Part I. Meteorology. J. Linn. Soc. Zool. Lond. *39.* 443.

BAKER, J. R. and BAKER, I., 1936. Part II. Botany. *39.* 507.

BAKER, J. R. and BAKER, Z., 1936. Part III. Fruit bats. Pteropidae. Ibid. *40.* 123.

BAKER, J. R. and BIRD, T. T., 1936. Part IV. Insectivorous bats. (Vespertilionidae and Rhinolophidae). Ibid. *40.* 143.

BAKER, J. R. and RANSOM, R. M., 1938. The breeding of southern hemisphere birds in northern hemisphere. Proc. Zool. Soc. Lond. *108.* 101.

BAKER, J. R., MARSHALL, A. J. and HARRISON, T. H., 1940. The seasons in a tropical rain forest (New Hebrides) Part V. Birds (Pachycephala). J. Linn. Soc. Zool. Lond. *41.* 50.

BASCOM, K. F., 1923. The interstitial cells of the gonad of cattle with special reference to their embryonic development and significance. Amer. J. Anat. *31.*

BISSONETTE, T. H., 1930. Studies on sexual cycles in birds. I. Sexual maturity, its modification and possible control in the European starling *(Sturnus vulgaris).* Amer. J. Anat. *45.* 289.

BISSONETTE, T. H. and CHAPNICK, M. H., 1930. Studies on sexual cycles in birds. II. The normal progressive changes in the testes from November to May in the European starling *(Sturnus vulgaris),* an introduced nonmigratory bird. Amer. J. Anat. *45.*

BISSONETTE, T. H., 1932. Modification of mammalian sexual cycles. Reaction of ferrets *(Putorius vulgaris)* of both sexes to electric light added after dark in November and December. Proc. Roy. Soc. Lond. *110.* 322.

BULLOUGH, W. S., 1951. Vertebrate sexual cycles. Methuen Monographs on biological subjects. London.

BUXTON, P. A., 1936. Breeding rates of domestic rats trapped in Nigeria and certain other countries. J. Anim. Ecology. *5.* 53.

CUNNIGHAM, I. J. and HOPKIRK, C. S. M., 1935. Dietary protein in relation to sterility. Newzealand J. Sci. Tech. *17.* 420.

DEANESLY, RUTH, 1935. The reproductive processes of certain mammals. Part IX. Growth and reproduction in the stoat, *Mustela erminea.* Phil. Trans. Roy. Soc. Lond. *225.* 459.

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GOPALAKRISHNA, A., 1949. Studies on the embryology of Microchiroptera. Part III. The histological changes in the genital organs and accessory reproductive structures during the sex-cycle of the Vespertilionid bat *Scotophilus wroughtoni* (Thomas). Proc. Ind. Acad. Sci. *30*. 17.

GROOM, G. R., 1933. The seasonal modification of the testis of vertebrates with special reference to interstitial cells. Proc. Zool. Soc. Lond. *103*.

— 1940. The seasonal modifications of interstitial tissue of the testis of the fruit bat, *Pteropus*. Ibid. *110*.

HILL, MARGARET, 1939. Reproductive cycle of the male weasel, *Mustela nivalis*. Ibid. *109*.

HOOKER, CHARLES W., 1944. The post-natal history and function of the interstitial cells of the testes of the bull. Amer. J. Anat. *74*. 1.

JAMESON, Jr. E. W., 1947. Natural history of the prairie vole *Microtus*. Univ. Kansas Publ. Museum of Nat. Hist. *1*. No. 7.

— 1953. Reproduction deer mice *(Peromyscus maniculatus* and *P. boylei)* in the Sierra Nevada. California. J. Mammalogy. *34*. No. 1.

KINGSBURY, B. F., 1914. The interstitial cells of the mammalian ovary *(Felis domestica)* Amer. J. Anat. *16*.

MARSHALL, A. J., 1947. The breeding cycles of an equatorial bat *Pteropus giganteus*. Proc. Linn. Soc. Zool. Lond. *159*. 103.

MARSHALL, F. H. A. and BOWDEN, F. P., 1934. The effect of irradiation with different wavelengths on the oestrous cycle of the ferret with remarks on the factors controlling sexual periodicity. J. Exptl. Biol. *11*. 409.

MEASROACH, VERONICA, 1954. Growth and reproduction in the females of two species gerbil *Tatera brantsi* (A. SMITH) and *Tatera afra* (GRAY). Proc. Zool. Soc. Lond. *124*. 631.

MEYER, BERT J. and MEYER, ROLAND K., 1944. Growth and reproduction of the cotton rat *Sigmodon hispidus* under laboratory conditions. J. Mammalogy. *25*. 107.

MILLER, ROLAND E., 1939. Reproductive cycle in the male bats of the species *Myotis lucifugus* and *Myotis griescens*. J. Morph. *64*. 267.

PRASAD, M. R. N., 1951. Changes in the reproductive organs of the male palm squirrel *Funambulus palmarum palmarum* (Linn). J. Mysore Univ. B. *12*. 89.

— 1954a. Natural history of the Indian gerbille *Tatera indica cuvierii* (Waterhouse) J. Bom. Nat. Hist. Soc. *52*. 184.

— 1954b. Food of the Indian gerbille *Tatera indica cuvierii* (Waterhouse). Ibid. 52. 321.

PERRY, J. S., 1945. The reproduction of the wild brown rat. Proc. Zool. Soc. Lond. *115*. 19.

RASMUSSEN, A. T., 1917. Seasonal changes in the interstitial cells of the testis of the wood-chuck *(Marmota monax)*. Amer. J. Anat. *22*. 475.

REGAUD, C.,* 1904. L'etat des cellules interstielles du testicule chez la taupe pendant la période spermatogenèse et pendant l'état de repos des canalicules seminaux. Compt. de. Rend. de. l. Assoc. d'Anat. T. *56*.

RICHTER, C. P., HOLT, L. E. and BARELARE, Jr., 1938. Nutritional requirements for normal growth and reproduction in rats studied by the self-selection method. Amer. J. Physiol. *122*. 734.

ROWAN, W., 1929. Experiments in bird migration I. Manipulation of the reproductive cycle: Seasonal histological changes in the gonads. Proc. Boston Soc. Nat. Hist. *39*. 151.

ROWLANDS, I. W., 1936. Seasonal changes in the reproductive organs of the male bank vole (*Evotomys glareolus* SCHREB). Phil. Trans. Roy. Soc. Lond. B. *226*. 71.

— 1938. Preliminary report on the reproductive cycles of the red squirrel *Sciurus vulgaris*. Proc. Zool. Soc. Lond. B. *108*. 441.

* Papers marked with asterisks were not available in original.

SESHACHAR, B. R., 1941. The interstitial cells of the testis of *Ichthyophis glutinosus* Linn. Proc. Ind. Acad. Sci. *13*. 244.

TANDLER, J. and GROSZ, S., 1911. Über den Saisonsdimorphismus des Maulwurfhodens. Arch. f. Entw. der organ. Bd. *33*.

VON BENEDEN, E.,* 1880. Contribution à la connoissance de l'ovarie des mammifères, l'ovarie du *Vespertilio murinus* et du *Rhinolophus ferrum equinum*. Arch. die Biol. T. *1*.

LESLIE, P. H., URSULA, VENABLES, M. and VENABLES, L. S. V., 1952. The fertility and population structure of the brown rat *(Rattus norvegicus)* in corn ricks and some other habitats. Proc. Zool. Soc. Lond. *122*. 187.

WELLS, L. J., 1935. Seasonal sexual rhythm and its experimental modifications in the 13-striped ground squirrel *Citellus tridecemlineatus*. Anat. Rec. *62*. 409.

WOOD, F. D., 1925. J. Mammalogy *16*. 105. quoted from Asdell's, Patterns of Mammalian reproduction. Comstock publishing company, Ithaca, New York.

WHITEHEAD, R., 1904. Embryonic development of the interstitial cells of Leydig. Amer. J. Anat. *3*.

* Papers marked with asterisks were not available in original.

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ON THE DIFFERENTIATION OF THE TOPOGRAPHY OF THE CRUSTACEAN HEAD

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1. INTRODUCTION.

During the present century diverging opinions were often expressed concerning the interpretation of the topography of the crustacean, and, on the whole, the arthropod head. These differences are well exemplified by the different morphological significance allotted to the labrum by various authors. If for the present we put aside the greater issue and confine ourselves to the labrum, the following possibilities stand open.

I. The labrum represents the original anterior end of the arthropod. This was recently maintained by SNODGRASS (1951), according to whom (l.c. p. 107) "the labrum is assumed to be the anterior pole of the primitive arthropod. The head region that carries the labrum, called epistome or clypeus, therefore is primarily facial or dorsal; the ventral position of these structures in most Crustacea, and in Trilobita and Xiphosurida, is due to their secondary inflection upon the under surface of the head. In amphipods, isopods, and insects the epistome (clypeus) is typically facial in position, dorsal or posterior to the labrum."

Along quite different lines of approach FERRIS (1942) and his collaborators, notably HENRY (1948 a,b) arrived at the opinion that the labrum represents the anterior end of Crustacea and Insecta.

ROONWAL from his embryological investigations on insects concluded that the acron has disappeared completely with the exception of the lobus opticus, and he regards the labrum as belonging to the first segment. The small importance attributed by him to the acron makes his point of view form a sort of transition to the theories dealt with in the next paragraphs.

II. The labrum does not constitute the original anterior end of the body but represents a simple or composite structure formed by one or more segments, possibly also with contributions from the ventral part of a true unsegmented acron.

HOLMGREN (1916), HANSTRÖM (numerous papers published between 1926 and 1934) and other writers from investigations of the nervous system in annelids and arthropods concluded that the arthropods possess a large prostomial acron, to which belong i.a. the antennulae. The eye region constitutes the anterior end of the arthropod, and the labrum, innervated from the tritocerebrum, belongs to the anteroventral part of the mouth region, which, in principle, is situated behind the prostomium.

When WEBER (1952 p. 41) writes that SNODGRASS founds his interpretation of the arthropod head upon the theories of HOLMGREN and HANSTRÖM, this should not be understood to include the anterior position of the labrum. It should also be noted that SNODGRASS in later years has changed his views on many problems connected with arthropod morphology and phylogeny. In 1951 (p. 5) he wrote: "The head characters of arthropods will be seen to be so variously distributed and combined in the several arthropod groups that they cannot be made to fit consistently into any theoretical scheme of arthropod interrelationships, and the writer hereby retracts whatever views on the subject he may formerly have expressed." To this statement, the outcome of the investigations during which SNODGRASS devoted his interest almost exclusively to the skeleton of the arthropod head, we shall have occasion to revert later on in this paper.

VANDEL (1949) apparently was inclined to accept the point of view of HANSTRÖM and HOLMGREN.

WEBER (l.c. pp. 51 ff.) tried to make a synthesis of the results achieved by various writers and especially the extremely careful investigations carried out by TIEGS (1940, 1947). He assumes that three originally postoral mesodermal segments (the pre-antennular, antennular, and antennar segments) advancing during the embryological development from behind the mouth all contribute to the formation of the labrum. It should be noted in this connection that the considerations of MANTON (1949), further developed by DAHL (1954), suggest the possibility that the ectoderm of the preoral region in the Crustacea is not

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primarily segmented and that a segmental arrangement is impressed upon it by mesodermal elements wandering forward from the blastopore margin.

Many other writers directly or indirectly have expressed views favouring either of the main standpoints outlined above. It would carry too far to give anything approaching a complete bibliography; the examples quoted above are sufficient to illustrate on the one hand how deep-set are the differences of opinion and on the other what a central position the interpretation of the labrum must assume in any theory aiming at a morhpological explanation of the topography of any arthropod head. Answers to several questions are obviously called for. Among the numerous points arising the present paper will deal mainly with the following ones.

1) The establishment of the theoretical anterior end of the crustacean body.

2) The influence of the mode of life in the widest sense on the morphological topography, and the tracing of ontogenetical changes in certain important topographical relationships.

3) The derivation of the labrum and its innervation.

4) The evaluation, as far as possible, of the interplay of phylogenetic, ethological and ecological factors in the establishment of the topography of the crustacean head.

The paper is based partly on studies of literature, partly on investigations of new material. The survey given of the modes of feeding of various Crustacea is based practically exclusively on the results of previous writers, for very good descriptions of the feeding habits exist for so many types of Crustacea that it was sufficient for the present needs. On the other hand the morphological and ontogenetical questions dealt with in the present paper were studied first hand on a large numbers of representatives of different Crustacean groups.

I am greatly indebted to Professor B. Hanström, Professor O. W. Tiegs and Professor K. G. Wingstrand for interesting discussions and kind advice. Professor C. H. Lindroth and Dr. P. Brinck adviced me on various entomological matters, Dr. F. H. Drummond, Melbourne, very kindly sent me specimens of *Koonunga cursor*. Mr. P. Ardö provided me with eggs of *Artemia* and *Triops*, and Miss Charlotte Holmquist collected Cladocera for me and permitted me to use series of sections of various Mysids from her own collection. Dr. K. Lindberg kindly determined the species of *Cyclops* included in my material. Miss Christine Blank gave valuable assistance with the technical side of the investigation.

2. THE ANTERIOR POLE OF THE CRUSTACEAN BODY

The establishment of the theoretical anterior pole of an arthropod is not an easy task. As already mentioned (p. 123) conflicting opinions on this point

were expressed by previous writers. Some of them, e.g. SNODGRASS, hold the distal end of the labrum to be the anterior end of the animal, others e.g. HOLMGREN and HANSTRÖM state directly or indirectly that the so called prostomial area i.a. carrying the protocerebral sense organs (eyes and frontal organs) is the real anterior end.

Three structural elements would appear to have a bearing upon the question implied, viz. the segmental mesoderm, the central nervous system, and the intestine. The drawing of conclusions from the exoskeleton would seem to be more dangerous, for the crustacean skeleton appears to be extremely adaptable to functional needs. This rather than the fruitlessness of phylogenetical discussions would seem to be the lesson to be learnt from the later investigations by SNODGRASS (1950, 1951, 1952). In particular it seems dangerous to base any conclusions solely on the conditions found among adults.

In a segmented animal the anterior end will obviously be the foremost one of a series of homodynamic segments or any unsegmented structure forming an anterior continuation of that series. It should be kept in mind that in the arthropods and also in the annelids (IVANOV 1928) the presence of mesoderm produced in two (or more) different ways strictly speaking deprives us of the right to consider segments with mesoderm derived from the anterior margin of the blastopore area as homodynamic with those formed from a proliferation zone or from teloblasts. But as the first type of segment in many species is formed before (in time) and anterior to the segments of the second type, and as the segments of the first type are, among themselves, arranged in a series, which forms an anterior continuation of the second series of segments, the criterion retains its validity.

In the Annelida the prostomium would seem to answer the definition of the anterior end, as given above. Even if, as suggested by RAW (1949), the stomodaeal part of the intestine in polychaets should represent a series of segments invaginated from the mouth region this does not affect the interpretation of the prostomium as a whole, for the invagination is considered to have taken place behind it, and the arguments for and against RAW need not concern us here.[1]

On the other hand certain statements by HENRY (1947 a, b) call for some comments. Like RAW but quite independently HENRY arrived at the conclusion that the eversible part of the polychaete intestine represents body segments. She was induced by her considerations of these matters to give the annelid

[1] After this paper had been completed another paper by RAW: "The Malacostraca: their origin, relationships and phylogeny" appeared in Ann. Mag. Nat. Hist. ser. 12 vol. 8 1955. It cannot be dealt with in detail but I wish to point out that I disagree completely with the suggestion made by RAW that various segments should have been drawn into the crustacean stomodaeum behind the labrum and that the ventricular armature of Malacostraca should represent vestiges of such segments or their appendages.

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prostomium a new morphological significance, and as it is extended to cover also the head of arthropods some attention must be devoted to it.

The ultimate object of Henry's investigation was to give a new interpretation of the morphology of the insect head, but in order to do so she reviewed most of the major annulate groups. Her conclusions are based exclusively upon the external topography of the nervous system of adults, a line of approach already sharply criticized by MANTON (1949) and WEBER (1952). Her method was dissecting under a "dissecting microscope". "Detailed points" were "verified by using histological sections." It seems to be a point of some interest that the fixative then used was 95 per cent alcohol (1947 a p. 66).

HENRY started from the Oligochaeta, and for this she gave the following reason (1947 a p. 68): "Some zoologists believe them (i.e. the Oligochaeta) to be a reduced form of the Polychaeta. However, the nervous system of the Oligochaeta represents the simple, clear, basic pattern for the nervous system as it is found in the rest of the Annulates."

In the course of her investigation she claims to have found that the oligochaete prostomium is, in fact, no prostomium, but the first true segment. No preoral structures exist in the Oligochaeta. "The mouth is morphologically apical and all structures are morphologically postoral." (l.c. p. 73).

Among the Oligochaeta HENRY laid particular stress on *Lumbricus terrestris* where the brain lies in the border area between the second and the third segment (third and fourth segments in HENRY's own enumeration where the prostomium is regarded as the first segment). Although she admits the secondary nature of this position as compared e.g. with that found in *Lumbriculus* it plays a considerable part in her considerations of the Polychaeta (1947 b pp. 86 ff.) and this is the reason why it has been mentioned here.

I shall not dwell here upon HENRY's description of the nervous system of *Lumbricus*, for I have not personally checked the facts presented there. I shall turn instead to her "objectice description" (1947 b p. 85) of the central nervous system of *Nereis* upon which she founds her interpretations of the morphology of crustaceans and insects. Concerning *Nereis* HENRY writes (1947 b p. 84) "... species of this genus present what seems to be the simplest and most readily understandable conditions of the nervous system that have been encountered in the Polychaeta in the course of this study. Had almost any other species been examined first, the problem might possibly have been dismissed as insoluble. Actually, the answers to the questions with which this work began are to be found in this genus without proceeding further. However, lest ist be urged that the conditions found in *Nereis* are, indeed, exceptional and represent merely coincidence it seems necessary to go further and to show that the conditions found in other Polychaetes are simple modifications of those found in *Nereis*. Therefore a considerable number of forms, representing several families, have been studied and from these a representative number are here conside-

red. We believe these to be sufficient to show that the conditions found in *Nereis* actually represent a basic type from which the other forms can be derived by processes of fusion of parts, loss of parts or change in the proportion of parts."

The assumption that *Nereis* should represent a basic type from which all others could be derived is against current ideas on Polychaete taxonomy. However, the main conclusions drawn by HENRY from her investigation of *Nereis* can be summed up in the following manner:

1) The innervation of the proboscis in *Nereis* can be homologized detail by detail with that of the prostomium and two anterior segments in *Lumbricus*.

2) The proboscis is not simply an eversible part of the alimentary canal, but represents the anterior part of the body, i.e. is homologous with the prostomium plus two anterior segments of *Lumbricus*.

3) The proboscis, consequently, is segmented and represents no less than three segments, viz. segment 1 consisting of the jaws and their immediate surroundings, segment 2, represented by the so-called maxillary ring, and segment 3, the oral ring.

4) What is generally recognized as the prostomium represents no prostomial structure but is a dorsal outgrowth from the third (oral) segment, and is referred to by HENRY as the cephalic lobe.

Fig. 1, taken from HENRY's paper (1947 b fig. 31) gives in diagrammatical form a clear concept of her views on *Nereis*.

As will be seen from fig. 1 the so-called first (jaw) segment is represented as receiving a nerve from the anterior ventral part of the brain. The "nerve of segment 1" and another nerve emerging from the upper part of the circumoesophageal connective both end in a large ganglion at the base of the jaw. In HENRY's drawing the "nerve of segment 1" is represented as the largest nerve leaving the brain. It seemed to me a remarkable fact that neither RETZIUS (1895) nor HOLMGREN (1916) in their investigations of *Nereis*, neither of which is referred to by HENRY, did find this large nerve. I therefore examined series of sections of the three species *N. virens*, *N. pelagica*, and *N. diversicolor*. The rather small nerve I in the nomenclature of HOLMGREN (l.c. p. 10) (= "first nerve" of HAMAKER 1898) which was noted also by HENRY and is supposed to run very close to the "nerve of segment 1", is easy to find, but of the latter, alledgedly much larger, there is no trace and I am fully convinced that no such nerve does exist. I am also convinced that what Henry saw was the strong tendon connecting the base of the jaw with the body wall. In specimens of *N. diversicolor* with extended pharynx it occupies almost exactly the same position as HENRY's "nerve of segment 1", and apart from the fact that it has no contact whatever with the brain it could, by he believing eye, be mistaken for a nerve (cf figs 2 and 3).

I also examined the so-called "secondary ganglion of segment 1", a large and conspicuous structure at the base of the jaw. Although some nerve fibres

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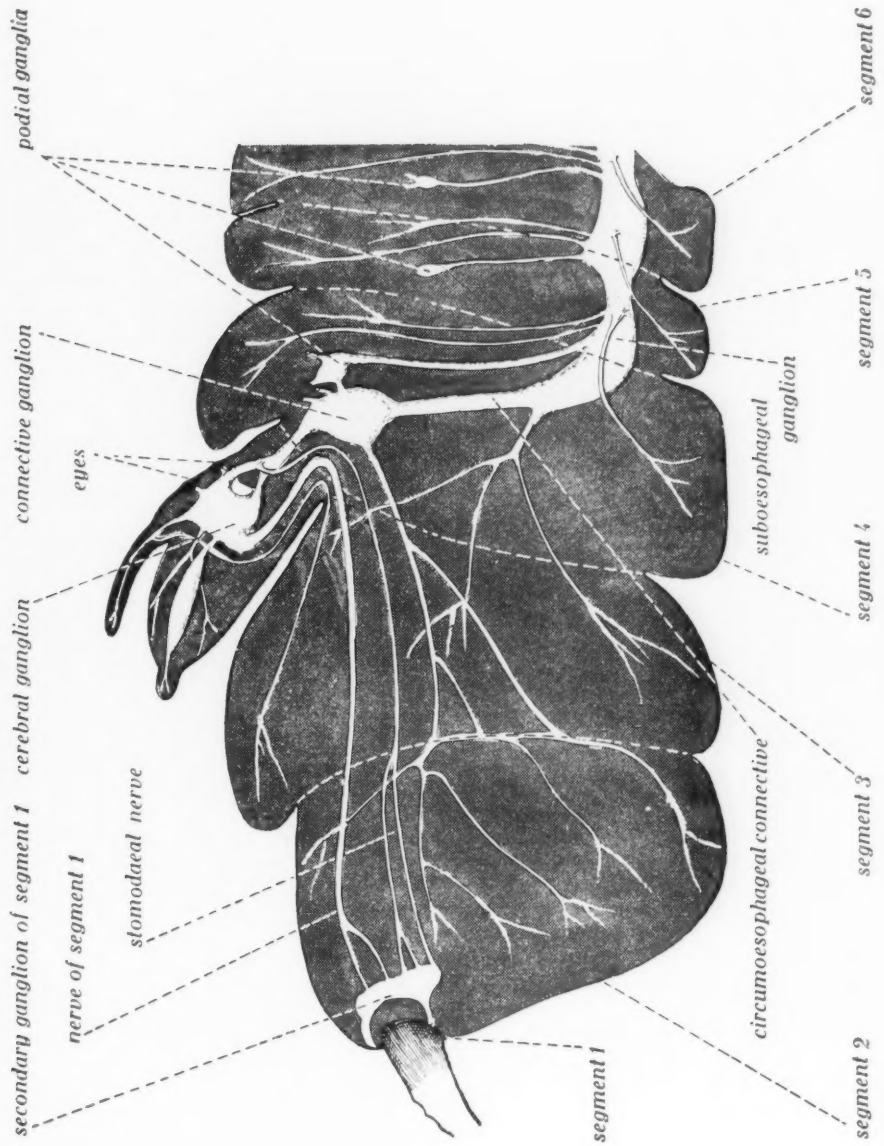

Polychaeta, Nereis virens, cephalic nervous system, lateral aspect

Fig. 1. The organisation of the head of *Nereis* according to Henry (1947 b).

probably enter it, it has got nothing whatever to do with a ganglion. It is in fact a dense mass of tendons with numerous nuclei. The tendons are attached with one end to the chitinous wall of the jaw, with the other to the muscles of the jaw apparatus (fig. 3).

It seems meaningless to pursue any further this scrutiny of details in HENRY's investigation of *Nereis*. What has been said in the last few paragraphs should be sufficient to show that the sceptical attitude assumed by MANTON (1949) and WEBER (1952) towards the theoretical foundation of her investigations should be extended also to her practical work. In fact her claims on morphological discoveries should not be accepted until checked by more critical investigators.

Concerning the views on the segmentation of the anterior part of the polychaete body put forward by HENRY, the following arguments could be advanced against them.

1) Nothing supports the assumption that the Oligochaeta should be more primitive than the Polychaeta or that their central nervous system should represent a basic annulate pattern any more than that of the primitive errant Polychaeta.

2) The choice of *Lumbricus* as a basic form upon which to found the discussion of the segmentation is entirely arbitrary and not supported by any facts mentioned by HENRY or otherwise known.

3) The same applies to the homologizations made by HENRY between the segmental innervation in *Lumbricus* or *Nereis*.

4) Among the Polychaeta *Nereis* does not constitute any particularly primitive type.

5) The description given by HENRY of the innervation of the proboscis in *Nereis* contains gross errors of observation.

6) Nothing favours the assumption that the prostomium ("cephalic lobe" in the terminology of HENRY) should be a dorsal outgrowth from any postoral segment.

In short, the identification of the polychaet prostomium with the anterior pole of the body remains unaffected by the arguments of HENRY.

This establishment of the prostomium as the morphological anterior pole of the Annelida is of primary importance also to the understanding of the topography of the arthropod head. For although all writers agree that the arthropods cannot be derived from any of the annelids of the present day there exists also, despite great differences of opinion as to details, general agreement on the point that the arthropods must be derived from some type of annelid ancestor. Consequently we have to reckon with the fact that the arthropods and their ancestors possess or once possessed a prostomium in direct anterior continuation of the series of body metameres and, like that of the present annelids, situated in front of the segment surrounding the mouth opening.

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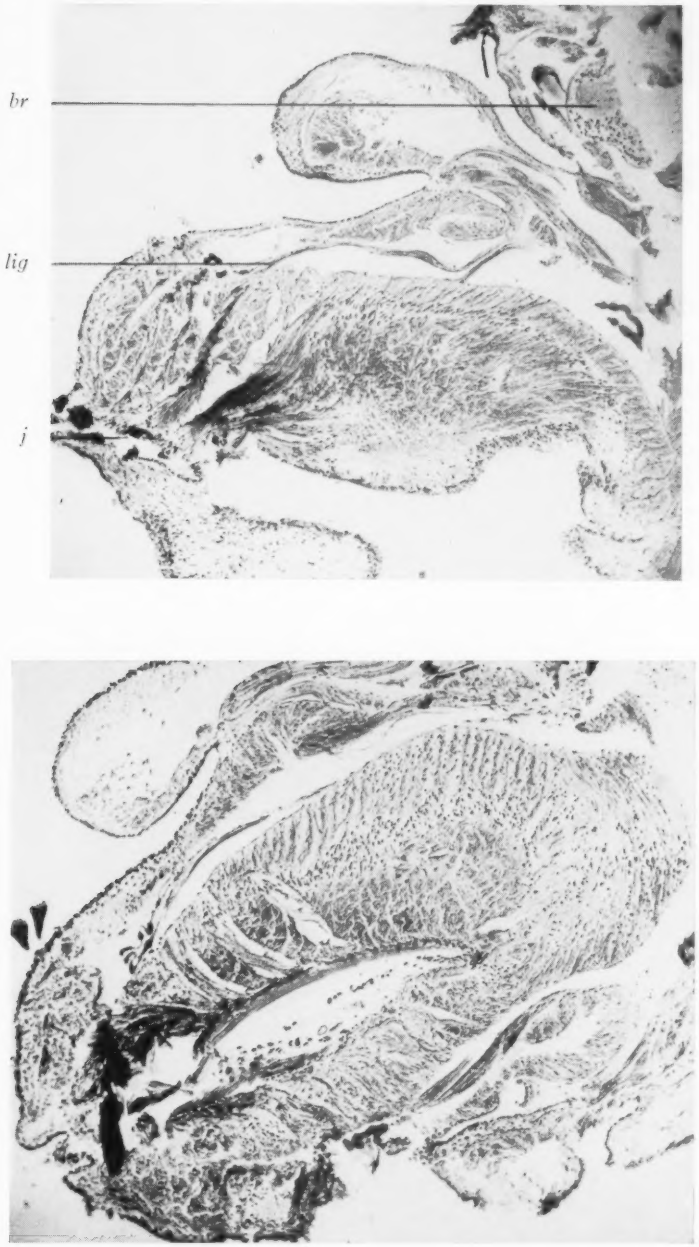

Fig. 2—3. Longitudinal section through anterior end of *Nereis diversicolor*. Silver impregnation according to Bodian. Note in fig. 2 (above) the strong ligament in a position exactly corresponding to Henry's "nerve of segment 1", and in both fig. 2 and fig. 3 the absence of any ganglion at the base of the jaw.

Especially the number and extension of the metameres in the anterior end of the arthropoda, or more particularly the crustacea to which the present discussion will be devoted, is the subject of widely diverging opinions. In the first place that is due to the type of mesoderm formation already referred to on p. 126. At present, however, the actual number of segments of the head need not concern us, for it has little direct bearing upon the establishment of the anterior pole itself. What is essential in this connexion is the fact that a series of mesodermal metameres continues from behind past the crustacean mouth opening and into the head proper (MANTON 1928, 1934). The same is the case in other groups such as the Onychophora (MANTON 1949), the Symphyla and the Pauropoda (TIEGS 1940, 1945, 1947). A formation of neuromeres also takes place, and although there exists no definite agreement between various writers upon the mutual relationships between the subdivisions of the mesoderm and the nervous system, the anterior end of the latter is obviously the part of the brain which innervates the eyes and the frontal organs, i.e. the protocerebrum, or at least the anterior part of it, and the protocerebrum forms a direct continuation of the series of neuromeres.

The area of the body surface where the sense organs innervated by the protocerebrum are situated does not necessarily coincide with the functional anterior pole of the moving custacean. This will be exemplified in later chapters.

The topography of the alimentary canal and its bearing upon the present issue will be the subject of the next chapter.

3. THE POSITION OF THE MOUTH OPENING IN RELATION TO THE LENGTH AXIS OF THE BODY

HANSTRÖM (1931 p. 141) called attention to the ventral position of the brain in certain Entomostraca and in other primitive arthropods such as the Xiphosura, probably also the Trilobita. LANG (1948 p. 108) pointed out that HANSTRÖM was mistaken in believing that the brain should lie ventrally in most Copepods. This remark by LANG is justified, but it does not affect the fundamentals of the statement made by HANSTRÖM, which is indeed an important one. As pointed out by HANSTRÖM the situation of the brain in a ventral position is a consequence of the inclination of the head in a ventral direction, found within the groups in question.

In most adult Annelida and also in the Onychophora this inclination of the head downwards is not to be found and the same to a certain extent applies to the position and direction of the mouth opening. (For a discussion of the position of the mouth during ontogeny cf p. 174). In many primitive Crustacea such as the Phyllopoda, the Mystacocarida and, among the Malacostraca, the

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Mysidacea the mouth lies ventrally and the mouth or at least the atrium oris has its opening directed backwards. The same applies to *Limulus* and seems to have been the case with the Trilobita. On the other hand in many higher Crustacea, particularly many Malacostraca, as well as in the majority of Myriapoda and Insecta the mouth opening again approaches, even if it does not fully attain the length axis of the body and opens as a rule more or less obliquely forwards-downwards. Here again we find the brain in a position dorsal to the length axis of the body. As we shall se later on, however, the presence of a (functionally) ventral mouth opening does not automatically postulate a ventral position of the brain; on the contrary obviously functional demands may produce adaptational deflections of the intestine and the central nervous system in opposite directions.

It lies near to assume that a dislocation of the mouth from the position probably found among the annelid ancestors to that encountered among the primitive arthropods was connected with functional changes. CANNON and MANTON (1927), CANNON (1928), and ERIKSSON (1934) suggested various ways in which food transport to the mouth by means of the appendages may have originated. It is highly suggestive that the most primitive Crustacea existing to-day feed by means of food currents produced by the appendages and entering the atrium oris from behind. The ventral position of the mouth and an atrium opening in a posterior direction are obviously essential to such a mode of feeding. In the next chapter the position of the mouth opening relative to the mode of feeding will be considered in some detail for most Crustacean groups.

Phylogenetically the dislocation of the mouth from a subterminal to a ventral position may have taken place in the following manner. The annelid (or annelid-like) progenitors of the arthropoda—the origin of which may well have been di- or even polyphyletic—most probably possessed a soft integument, and also most probably a subterminal mouth opening. It is true that certain recent polychaetes have the anterior part of the intestine bent down so that the mouth lies on the (functional) ventral side with the brain and prostomium anterior to it, but in the majority of annelids the mouth is subterminal and directed anteriorly. Possibly these ancestors had also an eversible pharynx. In any case we have the right to assume that their bodies possessed a high degree of flexibility, permitting the anterior end with the mouth opening to be moved in various directions without affecting the position of the body as a whole. The gradual development of the parapodia (or similar appendages) into arthropod limbs, perfected for swimming or walking, was at least partly reponsible for the formation of the arthropod exoskeleton, the higher efficiency of the locomotory apparatus demanding a more rigid fixation of the musculature (cf. SNODGRASS 1938). But the increasing rigidity of the integument brought about a corresponding decrease in the general flexibility of the body, creating the need of a new apparatus for the intake of food. Moreover no arthropod, fossil or

recent, is known to possess an eversible pharynx. However, a most interesting analogy with the eversible, armed pharynx of various polychaetes is provided by the stomodaeal armature of certain acephalic dipter larvae (fam. Syrphidae et al.) No segmental mouth appendages are present, but in the entrance to the fore-gut a fairly complicated chitinous armature exists, provided with articulated teeth which are used for the capture of prey.

To revert to the early arthropods, the obvious answer to the new demands created by the increasing rigidity of the integument lay in the adaptation of some or all of the segmental appendages to the transport, sifting and mastication of food particles. It also called for close topographical relationships between the mouth opening and the appendages included in the feeding mechanism. The simplest and most effective adaptation would seem to be a dislocation of the mouth to the functional ventral side of the body, and the available evidence indicates that this actually is the solution which was adopted, for, as already mentioned, we find the mouth in such a position in all the most primitive Crustacea, in the Trilobita, the Merostomata and various of the systematically dubious Burgess Shale fossils.

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The dislocation of the mouth opening from a more or less subterminal to a more or less ventral position cannot be effected without a bending of the axis of the alimentary canal, and it is a matter of the first importance to the interpretation of the topography of the arthropod head to know how this bending was effected. Unfortunately the question can only be approached indirectly by means of the ontogeny and comparative anatomy of recent annelids and arthropods.

Investigations of a considerable number of polychaetes and oligochaetes have shown that the formation of the anterior part of the alimentary canal in the two groups takes place, on the whole, in the following way (for a summary of literature with numerous references cf. KORSCHELT and HEIDER 1936 pp. 346 ff, 368 ff, 373 ff., further references below). The blastopore does not close completely. A small opening remains at its anterior end, and there "where the entoderm cells were last seen on the surface" (MEAD 1897 p. 250 on *Amphitrite*) the stomodaeum is formed from the ectodermal cells in the area of the last vestiges of the blastopore. Thus the stomodaeum is formed in the immediate neighbourhood of the anterior end of the blastopore, which itself corresponds to the definite anterior end of the midgut. The fusion between stomodaeum and midgut appears, at least in many genera, to take place just at the anterior end of the latter.

In the trochophora the midgut is generally bent so as to form an angle of more or less than 90°. During metamorphosis and by means of allometric growth afterwards this bending becomes nearly or completely straightened out so that the midgut of the adult is straight or nearly straight with the ectodermal part forming an anterior prolongation. In many sedentary polychaetes as well

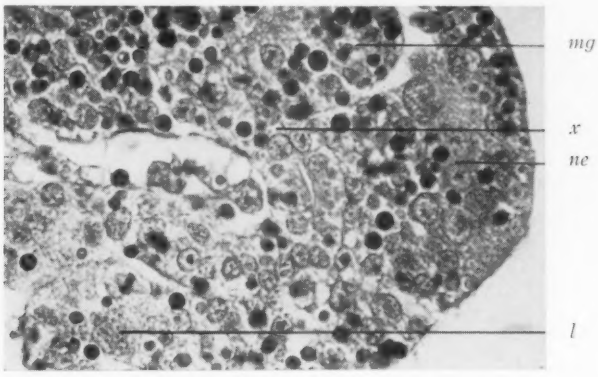

Fig. 4. Longitudinal section through embryo of *Artemia salina* showing the still closed inner end of the stomodaeum in contact with the wall of the mid-gut rudiment on the ventral side behind the anterior end of the latter.

as in echiuroids and sipunculoids the secondary coiling of the midgut obscures this simple pattern.

In the Crustacea matters are a good deal more complicated. The blastopore closes completely and it takes some time before proctodaeum and stomodaeum are differentiated. In the mean-time the definite ground-plan of the animal is, on the whole, attained. This means i.a. that the midgut becomes greatly extended in a frontal direction. When, in numerous Crustacea, the mouth is formed comparatively far back on the ventral side this will mean that it lies a good deal behind the anterior end of the midgut. It is therefore very interesting to learn whether the stomodaeum becomes connected to the anterior end of the endodermal gut or to the ventral side behind it.

It is not my intention to give any complete summary of previous investigations, but as the evidence is, perhaps, somewhat contradictory some instances from various groups will be given below.

Among the Euphyllopoda the ontogeny of *Artemia* was studied by FRÄNSENMEIER (1939) and recently also by myself. FRÄNSENMEIER devoted no particular interest to the present question but her figs. 1 and 3 (pp. 444 and 448) show the connection between the stomodaeum and the midgut on the ventral side of the latter some distance behind its anterior end. This was borne out by my own investigations (cf. fig. 4). In the free-swimming but recently hatched metanauplius, where the intestine has aquired a lumen, the same topographical relationships are retained. The same applies to young metanauplii of *Triops cancriformis* (fig. 6). In later stages the transformations befalling the anterior parts of the midgut to a certain extent obscure this arrangement, and the connection between stomodaeum and midgut lies very near the anteroventral corner of the medial part of the midgut.

The Cladocera are, apparently, different. KUHN (1913 pl. 17 fig. 80) has drawn the formation of the stomodaeum in *Polyphemus* at an early stage when it lies definitely in front of the solid strand of cells due to form the endodermal gut and with its lumen curving slightly backwards so as to meet the anterior end of the latter. In *Holopedium* the observations of AGAR (1908) imply that the blastopore should not close completely but that the mouth opening and stomodaeum should develop in direct connection with its anterior end and with the anterior end of the mid-gut, just as is the case in numerous annelids. As far as the open blastopore is concerned, this statement would seem to need confirmation. On the other hand the opening of the stomodaeum into the anterior end of the midgut seems probable enough, and conforms with my own observations on *Daphnia longispina*. The whole formation of the midgut in the Cladocera differs strikingly from that of the Euphyllopoda, for in the latter group it is very spacious, filling most of the body and having its dorsal wall bordering on the body wall, while in the Cladocera it forms as a narrow canal near the ventral side, and only in the area where it meets the stomodaeum does it approach the dorsal side.

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Nothing is known about the ontogeny of the alimentary canal in the Mystacocarida, but in the adult the ectodermal fore-gut is very long, forming a wide curve through the posterior part of the head region and meeting the anterior end of the endodermal mid-gut in the anterior part of the thorax (DAHL 1952). It seems highly probable that this represents essentially the original relationships between mid-gut and stomodaeum within the group.

From the investigations published so far it is difficult to form a definite opinion concerning the formation of intestine and stomodaeum in Copepods. FUCHS (1914 p. 136) and JACOBS (1925 p. 492) have shown that in *Cyclops* the blastopore closes completely after gastrulation. According to URBANOWICZ (1884 p. 616) the stomodaeum in Cyclops forms at the anterior end of the embryo, which might imply that it joins the mid-gut at its anterior end. In adults of *Harpacticus, Tigriopus,* and *Cyclops* the stomodaeum opens into the anteroventral part of the midgut, but in *Diaptomus* the junction lies a little further back on the ventral side.

The formation of mid-gut and fore-gut in the Ostracod *Cypris incongruens* was described by MÜLLER-CALE (1913 p. 146 and pl. 12 figs. 58—60). The blastopore closes completely. The midgut forms as a small vesicle a little below the centre of the body of the embryo, and the stomodaeum grows inwards from straight below it, i.e. from the centre of the ventral surface. The inward growth of the stomodaeum continues until it joins the ventral pole of the mid-gut vesicle. Later on (l.c. fig. 60) by means of allometric growth the mouth opening is pushed forwards and the part of the mid-gut vesicle joined to it is pulled along with the stomodaeum so that it appears to become the anterior end of the mid-gut.

The embryonic development of the Cirripedia was treated by various authors. In the present connection the very detailed investigations on *Balanus balanoides* carried out by DELSMAN (1917) are of particular interest. Some time after the complete disappearance of the blastopore an ectodermal invagination is formed (l.c. pp 504 ff), which gives rise to both stomodaeum and proctodaeum. Later on, the embryo becomes much more elongated, and proctodaeum and stomodaeum move apart, the latter approaching the anterior end of the body. At the last stage figured by DELSMAN (l.c. pl. 28 fig. 58) the inner end of the stomodaeum is pressed against the wall of the mass of yolk cells some distance behind its anterior end. However, at this stage the entodermal cells are not yet differentiated so as to form an intestinal epithelium, there is still no mid-gut lumen present, and the inner end of the stomodaeum itself is also closed. Therefore we do not know for certain at which point the definite junction between fore-gut and mid-gut takes place, but it is interesting to note that the inner end of the stomodaeum in the course of development passes along the ventral side of the future mid-gut.

Numerous investigations on Malacostraca (e.g. MANTON 1928 and 1934 on *Hemimysis* and *Nebalia*, REICHENBACH 1886 on *Astacus*, TAUBE 1915 on *Meganyctiphanes* and other euphuasids, HICKMAN 1937 on *Anaspides*, DELLA VALLE 1893 on *Talorchestia deshayesii* and other amphipods) all show that the junction between stomodaeum and mid-gut takes place at the anteroventral corner of the latter or further back on its ventral side. As a rule the anterodorsal part of the mid-gut projects further forwards than the anteroventral corner, and in several groups an anterodorsal diverticle is formed which projects forwards far beyond the junction between ectodermal and endodermal gut.

In summing up the discussion from the last few pages, we are permitted to draw the conclusion that in most Crustacea the stomodaeum joins the mid-gut not terminally as is apparently normal in annelids but subterminally or even a considerable distance away from the anterior end of the mid-gut. The Cladocera are a notable exception, for among them it appears probable that the junction lies at the anterior extremity of the mid-gut and possibly the same holds for Mystacocarida and certain Copepods. In some other cases conditions are not sufficiently clear to permit definite conclusions. About the general tendency, however, there can be little doubt. It seems probable that among the Crustacea the dislocation of the mouth in a ventral direction was brought about (or facilitated by) the complete closing of the blastopore and the severing of the connection between the invaginated entoderm and the ectoderm forming the body wall of the embryo. The direct contact between the ectodermal and endodermal parts of the gut is established late and not inherent in the very gastrulation as is the case with (at least numerous) annelids.

This state of affairs naturally throws grave doubts upon numerous supposed homologies between annelids and arthropods. Above all this applies to the much-

debated question of the homology between annelid and arthropod prostomium. When considering the different way in which mouth opening and stomodaeum are being formed in the two phyla, we have no right to assume whitout producing acutal proof that the portion of the body lying before the mouth should be the same (cf. discussions on pp. 179 ff).

For our discussion of the axis systems of the Crustacea it is an important point that the axis of the alimentary canal has got a break in its course. Obviously also the traditional definition of the ventral side as the area between mouth and anus, while adequate for the annelids, is not altogether a happy one in the case of the arthropods.

4. TOPOGRAPHY OF CENTRAL NERVOUS SYSTEM AND ALIMENTARY CANAL AS COMPARED WITH MODE OF LIFE

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It will be the aim of the present chapter to review, against the background of the previous discussions, the relative positions of atrium oris, mouth opening, stomodaeum, and central nervous system in various species where the mode of life is more or less well known. Special attention will be paid to feeding habits and to the position of the body when moving or resting. One or more representatives of most major groups of Crustacea will be dealt with.

a. BRANCHIOPODA

The normal way of feeding among Euphyllopoda as well as Cladocera is by filtering food particles from a current caused by the movements of the trunk appendages. Typically the mouth opening or in any case the opening of the atrium oris is directed backwards and lies close to the thoracopods. It does not matter in this connection whether the animal feeds in a normal position or lying on its back, for the important thing is the relationship between mouth and appendages. It should be noted, though, that in the theories about the origin of filter-feeding in arthropods elaborated by ERIKSSON (1934 p. 270) the position of the ventral side of the body close to the bottom is given a certain importance. It is worth nothing that the Devonian genus *Lepidocaris* in the position and direction of the mouth opening apparently resmbled the recent phyllopods (SCOURFIELD 1926). The rapacious non-filtering Cladocera (e.g. *Leptodora* and *Bythotrephes*) show interesting deviations from the ordinary pattern, which will be discussed in somee detail on p. 144.

Detailed descriptions of the mode of feeding in various Branchiopoda will be found in papers by CANNON (1922, 1929), ERIKSSON (1928, 1934), LUNDBLAD (1916, 1920), and STORCH (1922, 1924, 1925, 1929). Differences of opinion concerning various details still exist, but the main principles are clear

enough. We need not concern ourselves here with the details of the very complicated mechanism, to the present purpose it is sufficient to state that the final phase of the food transport towards the mouth in Anostraca, Conchostraca and most Cladocera takes place by means of a current flowing forwards in a groove along the ventral body wall. When approaching the opening of the atrium the food particles are taken care of by the maxillae and mandibles. In the Notostraca, living from considerably larger food particles than the types mentioned above, the forward food transport in the ventral groove takes place not by means of a current but by the active aid of the gnathobases on the thoracopods. *Leptodora, Bythotrephes* and possibly *Polyphemus* among the Cladocera seize and devour comparatively large animals. According to Borg (1935) the cladoceran genus *Anchistropus* is a true ecto-parasite of *Hydra*. With its three anterior pairs of thoracopods it scratches off small pieces of epithelium, which are, however, carried to the mouth in the normal way by means of a food-current.

In comparison with the very uniform mode of feeding within the group and the fairly stereotype arrangement of mouth opening, atrium oris and labrum, the amount of variation in the topography of the central nervous system stands out as rather striking. About the ventral nerve chord behind the mandibular region little enough is to be said, its course is, on the whole, closely parallel to that of the intestine; a few exceptions will be mentioned below. On the other hand the anterior part of the nervous system, from the mandibular region forwards, varies a great deal.

In the adults the most striking feature is the close topographical relationship between the brain and the cerebral sense organs, particularly the eyes. These sense organs are, undoubtedly, highly adaptable with respect to their location and many details in their structure.

In the Anostraca, swimming back downwards, the compound eyes are protruding on their stalks from the lateral sides of the head, their visual elements being arranged so as to permit a wide field of vision, the main axis of which is roughly perpendicular to the length axis of the body. The nauplius eye lies on the dorsal side very close to the (functional) anterior end of the animal, and in the normal swimming position it is directed obliquely forwards-downwards.

Ontogenetically the topography of the nervous system in *Artemia* can be outlined as follows. In the embryo still within the egg membrane the rudiment of the nervous system follows the contour of the ventral margin of the body, and consequently forms an arch, the anterior extremity of which is the optic ganglion of the nauplius eye which occupies the extreme anterior end of the body. Later on in connection with the stretching of the body which takes place approximately at the time of hatching the curve is straightened out so that the whole system up to and including the deutocerebrum forms a straight line with the optic ganglion of the nauplius eye in front and slightly above the

Fig. 5. Sagittal section through anterior end of *Limnadia lenticularis* orientated as in normal position.

rest, and the early rudiments of the compound eyes and their ganglia occupying lateral positions above the anterior end of the main system. This is, on the whole, the pattern retained in the adult.

The conchostracan *Limnadia lenticularis* moves along the bottom. When swimming its normal position according to LUNDBLAD (1916 p. 253) is back upwards, although its balance is precarious, so that it turns over easily. When feeding it moves slowly along the bottom, stirring the deposit and filtering edible particles. Behind the maxillary region in *Limnadia* the body is bent downwards almost at right angles. This bending befalls both the intestine and the ventral nerve chord, which follows it closely. The brain lies in direct continuation of the ventral chord, its position therefore is decidedly ventral. Only the compound eyes, which have fused along the medial line, are bent up in a dorsal direction. When the crustacean is moving over the bottom back upwards, the complex eyes are situated at the functional anteroventral corner of the body. Their main axis is directed forwards, but their ommata are arranged so as to give a field of vision covering all directions. The nauplius eyes lie on the (functional) ventral side, a little behind its anterior end. Owing to the bending

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in the maxillary region already referred to, the arrangement is, however, different when considered from a morphological point of view. Morphologically the compound eyes occupy the anterior end of the body and their axis points dorsally, while the nauplius eye is situated at the real anteroventral corner of the body. Thus the bend behind the mouth, which is probably favourable from the point of view of the feeding apparatus, is compensated for by another bend at the base of the compound eyes which gives the latter an unimpaired field of vision forwards, towards the sides, and upwards. Thus the same result is attained as in the Anostraca, with their lateral compound eyes. Laterally situated eyes would seem less suited to the needs of the Conchostraca owing to the difficulties caused by the bivalve shell (for further data on the nervous system of *Limnadia* cf. HANSTRÖM 1931).

The Notostraca spend most of their time resting or moving on the surface of the bottom with the back upwards. When swimming they assume varying positions, but mostly they retain their normal position, a fact which I have been able to check on numerous specimens of *Triops cancriformis* reared in the laboratory.

The compound eyes are situated dorsally, and the same is the case with the nauplius eye and the frontal organs (for details on the brain and sense organ of the Notostraca cf. HOLMGREN 1916, HANSTRÖM 1928 and 1931). The anterior part of the central nervous system has assumed a corresponding position, being bent upwards at almost right angles from the ventral nerve chord. In a specimen of *Triops cancriformis* 8 mm in length, the area of inflexion lies in the anterior part of the connectives between the tritocerebrum and the deutocerebrum. In full-grown specimens of *Lepidurus* (HOLMGREN l.c. figs 20—21 pp. 124—125) and *Triops* (own observations) the inflexion lies even further backwards, in the tritocerebral-mandibular area. Thus we find that in the Notostraca a mode of feeding consisting of the more or less automatical selection of fairly large food particles from currents caused by the thoracic appendages and needing a ventral mouth and an atrium oris opening backwards is combined with dorsal protocerebral sense organs designed to suit the benthic mode of life. The result is that the length axis of the brain is parallel with that of the stomodaeum but that they run in opposite directions.

An examination of the ontogenetical processes leading up to this arrangement is elucidating and will be briefly dealt with below (cf. figs 6—7, also CLAUS 1873 pp. 118 ff.).

I have had acess to no embryos of *Triops*, but in young metanauplii a few hours after hatching the central nervous system constitutes an almost straight line (fig. 6). The nauplius eye-frontal organ complex lies at its anterior end, forming a direct and practically straight continuation of its main axis. The rudiments of the compound eyes branch off from this main axis a little behind the nauplius eye centre. At this stage, the compound eyes, too, definitely belong

Fig. 6. Sagittal section through recently hatched metanauplius of *Triops cancriformis*. Note the position of the brain and the opening of the Stomodaeum behind the anterior end of the midgut.

Fig. 7. Sagittal section through brain of young *Triops cancriformis* about 8 mm. in length. Note the great topographical changes in comparison with the young stage in fig. 6.

to the ventral side of the body, for the axis of the compound eye rudiment forms a very acute angle with the main axis of the central nervous system.

In later stages it is possible to trace how, step by step, the anterior end of the body with both nauplius eyes, compound eyes and frontal organs is gradually bent over upwards and backwards until the sense organs assume the apparently dorsal position characteristic of the adult, while simultaneously the central nervous system is also being bent in the manner described above (fig. 7). The antennulae, the relative importance of which is rapidly diminishing, remain in a ventral position.

It is interesting to note that the early metanauplius is a freeswimming organism with a functioning nauplius eye but with the compound eyes still very rudimentary. When swimming the metanauplius orientates its body in many different ways, but very often with the back downwards or obliquely downwards, when, as often happens, the anterior end is kept a little above the

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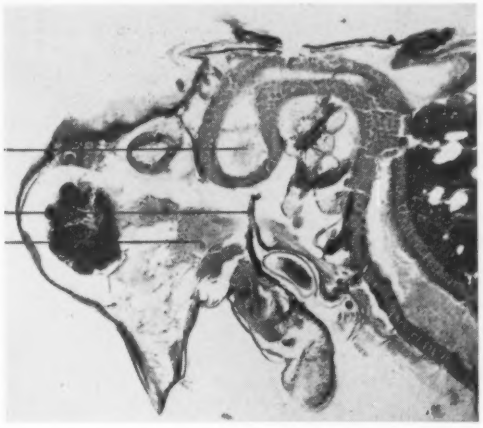

Fig. 8. Parasagittal section through *Daphnia longispina*.

posterior end. Sometimes, and especially when disturbed, it lets itself sink to the bottom and rests there for a while. In a number of instances observed by me on three or four different larvae, the metanauplius when thus resting was always lying on its back. At this stage then, the nauplius eye is generally directed upwards or upwards-forwards, towards the surrounding water and away from the bottom. It is interesting to note that the resting position of the larvae is opposite to that of the resting or feeding adult and that in both cases the most effective visual organ is directed upwards.

The interplay between the mode of life, the position of the cerebral sense organs, and the topography of the nervous system seems very obvious in the Notostraca. On the whole the three main groups of Euphyllopoda, viz. the Anostraca, the Conchostraca, and the Notostraca produce a highly suggestive illustration of the way in which the mode of life in general by means of a long-time selection affects the structural plan of various organ systems.

Concerning those Cladocera, which have a feeding mechanism of the ordinary type, it may be sufficient with a brief discussion of *Daphnia longispina*, which species I have studied morhpologically. The anterior part of the midgut forms, together with the stomodaeum, a double bend, so that its contour comes to resemble that of an inverted U. The ventral nerve chord, however, does not follow the outline of this U but cuts across its open end, near to the ventral body wall. This applies not only to adults but also to comparatively large young in the dorsal brood-pouch. In embryos, however, where the intestine is but recently formed, the U does not exist in anything approaching its final shape; the anterior part of the midgut leaves the ventral side of the body and curves slightly upward to meet the inner end of the stomodaeum, that is all. At this stage the nerve chord much more closely follows the course of the intestine,

without, however, to leave its ventral position. What happens later on is apparently an increase in relative length of the intestine which brings about a slight coiling, while there is no corresponding increase in length of the central nervous system.

Concerning the position and function of the sensory organs, particularly the eyes of *Daphnia*, valuable observations were published by WOLTERECK (1913). The main function of the compound eyes in a holopelagic genus like *Daphnia* is to regulate the position of the animal relative to the light. As pointed out by WOLTERECK they act rather as static than as visual organs in the stricter sense. The few ommatidia are arranged so that the eye can react upon light-beams falling in from any direction. The fused compound eyes and their unpaired optic centres form an anterior elongation of the central nervous system. This means that when the *Daphnia* is swimming in its normal position, i.e. with the length axis of the body tilted somewhat forwards from the vertical plane the compound eye is turned more or less obliquely towards the surface and the main source of light and is not obstructed by the body. The nauplius eye lies on the ventral side of the brain.

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The rapacious Cladocera of the genus *Leptodora* and the family *Polyphemidae* (*Bythotrephes, Polyphemus*) resemble each other in having the slender thoracopods arranged so as to form a basket ("Fangkorb") in which the prey is captured. Its wide opening is directed forwards. WESENBERG-LUND (1937, p. 443) in principle compares it with the catching apparatus of the Odonata. In other respects, however, the *Leptodora* and the polyphemid types differ widely from each other.

The polyphemid head does not differ so very much from that of the Cladocera in general. The atrium oris opens in a ventral direction behind a large and prominent labrum, the stomodaeum is short and at least in *Bythotrephes* it is perpendicular to the mid-gut, a good deal behind its anterior end. The prey (other crustacea) is chewed by the mandibles and swallowed. The anterior part of the central nervous system in *Bythotrephes*, from the ventral chain behind the mouth forwards, is straight, and the large compound eye forms the anterior termination of the system. Ommatidia are directed downwards, forwards, and upwards to just behind the vertical plane and also towards the sides. In a sagittal section through the median plane the ommatidia form a little more than a semicircle. Those directed upwards are particularly large. The nauplius eye is lacking.

In *Leptodora* the head is transformed so as to be almost monstrous. The thoracopods are drawn forwards and lie very close behind the mouth opening, which latter lies almost in the bottom of the "Fangkorb". The stomodaeum opens into an atrium oris which is directed ventrally behind a rather large labrum. The stomodaeum is very long and joins the anterior end of the mid-gut very far back, in the abdomen. It is also very narrow, and according to WESEN-

BERG-LUND (1937 p. 443) it seems probable that *Leptodora* sucks out its prey. The anterior part of the stomodaeum runs in a dorso-ventral direction, but after that it turns abruptly away in a caudal direction.

The most peculiar shape of the head in *Leptodora* is reflected in the topography of the nervous system, described by HANSTRÖM (1931 pp. 133 ff. with figs., particularly elucidating is the diagram fig. 27 p. 135). Some distance behind the mouth and in front of the large ganglion formed by a coalescences of the individual ganglia corresponding to the thoracopods the main axis of the central nervous system—like that of the whole body—is bent ventrally. From the point of inflexion forwards the nervous system is straight and, when the *Leptodora* assumes its natural position in the water, it lies horizontally. The compound eye forms the anterior termination of the whole nervous system. Its ommatidia are pointing in all directions and are absent only where the optic nerve enters the eye from behind. A nauplius eye is said to be present only in specimens hatching from the winter eggs.

b. MYSTACOCARIDA

The Mystacocarida probably filter their food from the water by means of the limbs in the maxillary region and the food enters the atrium oris from behind (cf. CHAPPUIS and DELAMARE DEBOUTTEVILLE 1953). In no other crustacean of the present day does one find such an extreme degree of separation of the pre-oral head region from the labrum and the mouth opening, and nowhere else does the posteriorly directed atrium oris open so far backwards. Proportionally the labrum is larger than in any other recent adult crustacean. The stomodaeum is very long and forms an arch of nearly 180° before it joins the anterior end of the mid-gut.

The nervous system forms a straight line without any deflection of the brain in a dorsal direction. The Mystacocarida live in the narrow spaces between the grains in marine sands, and their eyes are very poorly developed and situated in the so-called "rostral plate" which forms the anterior end of the body and has nothing whatever to do with a rostrum in the ordinary sense (DAHL 1952). On the other hand the antennulae are very large and extended forwards. Consequently the main sensory apparatus is designed to receive stimuli from the interstitial spaces between the sand-grains in front of the animal. It seems probable that this accounts for the peculiar shape of the head where the main sensory centres of the brain are pushed far forwards. It seems probable, then, that adaptational changes account for a good deal of the remarkable features found in the morphology of the Mystacocarida. On the other hand it seems probable that the retention of various very primitive traits have contributed considerably to the final result.

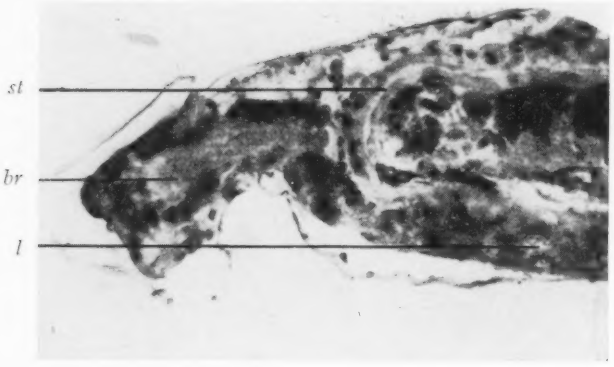

Fig. 9. Sagittal section through anterior end of *Derocheilocaris galvarini*.

c. COPEPODA

Among the free-living Copepoda—the parasitic forms will not be considered here—there exists a certain degree of variation with respect to the topography of the brain and sense organs, the oral area, and also the mode of feeding.

The planktic Calanoida are generally filterers. This was proved by STORCH and PFISTERER (1925) for *Diaptomus* and by CANNON (1928) for *Calanus* and *Diaptomus* while BREHM (1927 p. 468) called attention to the presence in *Acartia, Centropages,* and *Temora* of a catching apparatus very similar to that of *Diaptomus*. Probably the results achieved by the writers referred to will be applicable to a very great number of genera.

The Calanoida retain a number of apparently rather primitive morphological features, e.g. in the structure of their mouth appendages. According to observations by CANNON (1928) the feeding current in *Calanus* and *Diaptomus* is a vortex caused by the movements of the mouth appendages, which forces the water through the setae on the medial side of the second maxillae, where food particles are caught and passed on forwards to the mandibles and into the mouth. The food thus reaches and enters the mouth from behind.

In *Diaptomus zachariae* where I studied the topography of the head region, the labrum is very large and the atrium oris points obliquely backwards, although the projecting hypostome tends to deflect its posterior opening a little. The central nervous system runs straight and parallel to the midgut past the stomodaeum and into the tritocerebrum. In the zone of transition between the tritocerebrum and the deutocerebrum its axis is deflected upwards, forming an angle of about 60° upon its former course. The position of the antennular bases is (functionally) ventrolateral, and the antennular nerves leave the brain in the deflection zone and go forwards into the appendages. The nauplius eye lies upon the (morphologically) ventral (functionally anterior) side of the brain and thus lies very near the functional anterior end of the body, and is orientated in

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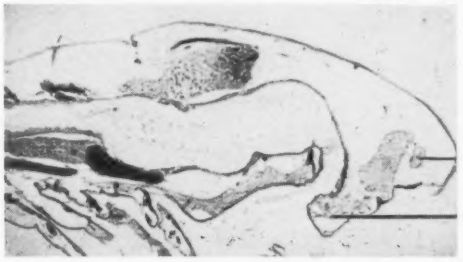

Fig. 10. Sagittal section through *Diaptomus zachariae*.

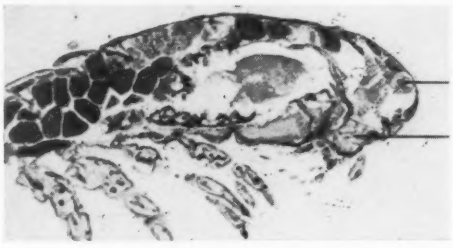

Fig. 11. Sagittal section through *Cyclops strenuus*.

the direction of movement. The nerves of the frontal organs are paired and go along the dorsal side of the head towards the frontal appendages (cf. DAHL 1953 pp. 2 ff.). Numerous further references to the topography of the nervous system in Calanoida will be found in RICHARD 1891.

Among the Cyclopida some genera at least are known to feed upon comparatively large objects. Some of them are predatory and catch small Crustacea. *Cyclops* is one such genus.

Cyclops strenuus has got a small labrum and the stomodaeum and the small atrium oris point obliquely backwards. As in *Diaptomus* the anterior part of the central nervous system is deflected upwards, but this is achieved in a slightly different manner, for in Cyclops one can see a very slight deflection upwards already in the maxillary-mandibular region, from where the central nervous system runs in a straight line, forwards and a little upwards, until a second and very marked bending takes place in the deutocerebrum. As in *Diaptomus* the antennulae are ventrolateral, and the nauplius eye is situated on the morphologically ventral side of the brain, near the functional anterodorsal end of the body.

The Harpacticida, mainly benthic in contrast to the types just dealt with, are more specialized with respect to various morphological features. LANG (1948) records no filterers among the benthic harpacticids, and he is of the opinion that the species living on algae feed mainly by grazing on diatoms and other small algae growing on the fronds of the larger species. From LANG's fig. 78 (l.c. p. 105) it can be seen that the stomodaeum of *Tigriopus fulvus* is directed

Fig. 12. Sagittal section through *Harpacticus chelifer*. Note in comparison with fig. 10 and 11 the different arrangement of the labrum and the lifting up of the ventral side in front of it.

ventrally and slightly forwards, and in the related species *Harpacticus chelifer* and *Zaus spinatus* investigated by myself, the same arrangement recurs, with the atrium oris slightly curved and its opening directed ventrally and placed far forwards, at least relative to the functional anterior end.

As in *Diaptomus* and *Cyclops* the brain in *Harpacticus* and *Zaus* is deflected upwards. The zone of inflection lies in *Harpacticus* in the transitional area between tritocerebrum and deutocerebrum, in *Zaus* further backwards, in the mandibular region. The nauplius eye and the frontal organs in *Harpacticus* and *Zaus* assume a position closely similar to that described for *Diaptomus* and *Cyclops,* although the nauplius eye is situated even closer to the dorsal surface of the head. But in *Harpacticus* and apparently generally among harpacticids, the arrangement of the anterior appendages differs from that found among cyclopids and calanids. As pointed out above the antennulae in both *Diaptomus* and *Cyclops* are ventrolateral in position. When the Copepods are in their normal position in the water the antennulae of both of them very definitely give the impression of belonging to the ventral side. But in *Harpacticus* the whole anterior part of the ventral surface of the body has become deflected upwards, so that the antennulae have become facial. When the copepod is in its natural position on the bottom the points of insertion of the antennulae assume a functionally anterodorsal position. Simultaneously the antennae become anteroventral. That it really is a deflection of the whole anterior end of the body rather than a dislocation of the appendages which has taken place is made probable by the orientation of the labrum. In *Diaptomus* and *Cyclops* the labrum is directed obliquely backwards, its longitudinal axis forming an angle of about 45° with that of the body. In *Harpacticus* on the other hand the longitudinal axis of the labrum is curved but on the average roughly perpendicular to that of the part of the body lying behind the mouth and the morphologically ventral margin of the labrum belongs to the "face" of the copepod.

Zaus, in principle, shows exactly the same arrangement. This is particularly interesting because of the dorsoventral flattening of the body which produces the impression that the whole ventral side of *Zaus* is pressed against the fronds of the algae upon which the species lives. However, a closer examination shows

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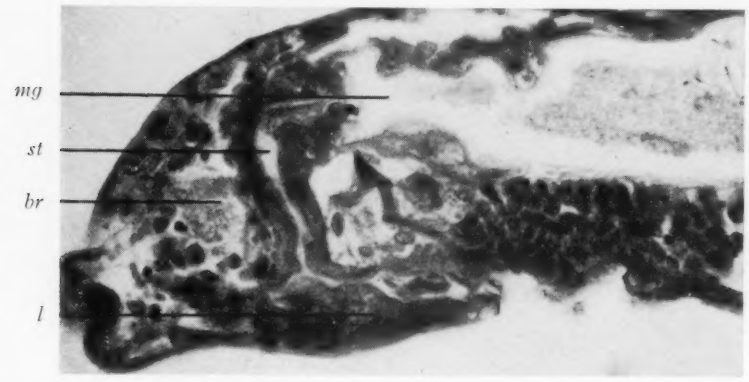

Fig. 13. Sagittal section through nauplius of *Harpacticus chelifer*. Note the considerable difference in the topography of the head region as compared with that of the adult specimen in fig. 12.

that this is not the case. The distal ends of the projecting labrum and hypostome are in contact with the substratum, but in front of the labrum the ventral surface of the body curves upwards just as in *Harpacticus,* although the rostrum and anterior margin of the cephalon by curving slightly downwards tend to mask this arrangement when seen from the dorsal side.

It seems obvious that such an arrangement of labrum and hypostome as that found in the bottom-living harpacticids dealt with here place the opening of the atrium oris and the mandibles in a position which is advantageous to an animal grazing or browsing on the bottom. The arrangement closely resembles that found by CANNON (1926) in the benthic ostracod *Pionocypris vidua.* (Cf. p. 151.) To the free-swimming forms, *Diaptomus* filtering its food from a current entering the oral area from behind and *Cyclops* catching its prey by means of postoral appendages, a posterior position and direction of mouth opening and atrium oris is well suited to the functional needs. But to the grazing form it seems equally desirable from a functional point of view to get the mouth opening located at the anterior end of the functional ventral side, where it can ingest the food most easily. In the harpacticids the turning up of the anterior part of the ventral side is probably an adaptation meeting this functional requirement. Simultaneously it brings the main cerebral sense organs, nauplius eye, frontal organs, and antennulae, into a position where they are best placed to receive stimuli from the area in front of and above the animal, i.e. from the directions most relevant to a benthic form.

A study of some of the ontogenetical changes throws further light upon the present questions. As far as I know no investigation of the anatomy of any harpacticid nauplius was previously carried out, and therefore I made series of sections nauplii and early copepodites of *Harpacticus chelifer.*

Harpacticid nauplii are generally dorsoventrally flattened (LANG 1948 pp. 143 ff.) with a comparatively large labrum and with the appendages attached to the ventral side. *Harpacticus* conforms to this type, in fact the only exception known seems to be the planktic nauplii of *Longipedia*, which are pear-shaped, and in this respect resemble those of the Calanoida (LANG l.c. p. 144).

The internal topography of the nauplii of *Harpacticus* differs in various important respects from that of the adults. The large labrum forms a thin plate which lies close to the ventral surface of the body, the hypostome which is so prominent in adults, is not yet in evidence. Consequently the narrow atrium oris opens straight backwards, and the mouth opening proper points obliquely backwards. The atrium plus stomodaeum runs forwards-upwards-backwards and thus completes an entire semicircle, or, rather, is twice bent at right angles, before joining the midgut. Diatoms apparently constitute the diet already at this stage, for there are plenty of fragments of diatom shells in the midgut and the proctodaeum, in the mid-gut together with a fine, granular, yellowish-brownish substance which is probably the half-digested contents of diatoms.

The central nervous system forms an almost completely straight line with only the slightest deflection upwards of the protocerebral part.

To avoid unnecessary handling of the delicate larvae set aside for sectioning I did not try to determine the exact stages but made sure that both early and late nauplii were represented. In practice this turned out to give satisfactory results, for with respect to the internal anatomy the early and the later stages are practically identical. During the transition from nauplius to copepodit, however, fundamental changes appear to take place, for an early copepodit already in all essentials represents the organization of the adult.

In summing up the present discussion it can be stated that the freeliving copepods within a morphological pattern incomparably more rigid than that of the Branchiopoda nevertheless show a number of interesting modifications. The available evidence seems to indicate that the ventral position of the central nervous system which is ontogenetically primary has also got a phylogenetical tradition within the group, even if it is probably not retained in any recent adult non-parasitic copepod. The position of the mouth opening comparatively far backwards, while excellently suited to the mode of life of many recent calanoids and cyclopids was probably also typical of the ancestors of recent copepods. This assumption is supported by the observations made on the nauplii of *Harpacticus*, i.e. a representative of the most specialized of the major types, for despite the fact that the nauplius like the adult feeds on diatoms the topography of its labrum, atrium oris, and stomodaeum is almost identical with that found in the very primitive Mystacocarida, which are probably not very far removed from the ancestors of the Copepods.

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d. OSTRACODA

Direct observations on the mode of feeding and critical discussions of the function of the oral appendages in ostracods were published by CANNON (1926, 1931, 1940).

In *Pionocypris vidua* CANNON (1926 pp. 328 ff.) could directly observe that food particles were kicked up from the bottom by the antennae. A current caused by the action of the vibratory plates on the mandibles and, above all, on the maxillulae enters the anterior end of the forward shell chamber, and with it the food particles are sucked in. The current is divided and passes on both sides of the large labrum which, with the hypostome, projects downwards as a mouth cone (cf. *Harpacticus* and other benthic harpacticids p. 147) almost to the ventral margin of the shell. In passing the labrum food particles are caught by the mandibular palps and by the interaction of the mandibles and the maxillulae as well as by the aid of the secretion from the labral glands they are brought into the mouth.

In *Cypridina (Doloria) laevis* CANNON (1931 p. 438) could conclude by inference that the mode of feeding is essentially analogous, food particles being also in that species drawn into the forward shell chamber by a current entering at its anterior end. In this case, however, food particles are probably stirred from the bottom not by the antennae but by the tips of the mandibles which can be projected beyond the edges of the shell valves.

In *Gigantocypris mülleri,* finally, CANNON (1940 pp. 193—194) concludes that the mandibular palps of the large and slow-swimming planktic species can be protruded through the antennal notches of the shell to catch the very active prey (*Sagitta,* copepods) upon which it feeds.

Thus the food enters the shell chambers of the investigated ostracods at or near the anterior end of the shell valves. The mouth and the atrium oris, on the other hand, are directed ventrally (*Pionocypris* and one *Candona* species investigated by myself) or a little forwards (*Doloria, Gigantocypris*). (For details cf. the admirable graphical reconstructions published by CANNON in the papers quoted above).

In front of the mouth the body is bent upwards in an even more marked manner than in the benthic harpacticids, so much in fact that the point of insertion of the antennulae often is not only dorsal but even posterior to that of the antennae. The nervous system is bent upwards in the same manner from an area of inflection generally in the mandibular region. The nauplius eye and frontal organs are functionally frontal in position, the compound eyes functionally dorsolateral.

e. CIRRIPEDIA

The sessile Cirripedia are not very relevant to the present discussion. A summary of the mode of feeding of the Thoracica was given by BROCH (1927

p. 529). Food particles are caught by the cirri and by their action transported forwards to the mouth parts in the oral cone, from where they enter the mouth. In *Lepas* the mouth opening is directed obliquely backwards (BROCH l.c. fig. 485 p. 516), and in *Lithotrya* this tilting backwards of the oral cone seems to be even more pronounced (CANNON 1947 fig. 5 p. 98). The anterior part of the central nervous system is somewhat deflected towards the dorsal side.

f. MALACOSTRACA

Leptostraca. The feeding mechanism and feeding habits of *Nebalia* were described in detail by CANNON (1928). The normal mode of feeding is by means of a current produced by the movements of the thoracic endopods. *Nebalia* generally lies buried just below the surface of the mud in which it lives. The "filter chamber" (CANNON l.c. p. 356) into which the food current enters, consists of a tubular space between the trunk limbs. The filter chamber is closed "dorsally by the ventral body wall, laterally by the proximal parts of the trunk-limb endopodites, and ventrally by the mat of long setae born on the recurved tips of the trunk-limbs." (CANNON l.c.). The entrance lies anteriorly between the rostrum, the eye-stalks, and the antennular bases. It can be closed by lowering the eye-stalks, shifting the position of the antennulae and bringing the rostrum downwards. The current thus enters the filter chamber at its anterior end and food-particles are strained off by the rows of setae on the trunk limbs and passed forwards to the mouth-parts by the gnathobasic setae. Larger food particles can be picked up from the bottom and conveyed to the mouth.

CANNON strongly emphasizes that the filter apparatus in *Nebalia* is unique and cannot possibly be derived from that of the *Anostraca* as was suggested by STORCH (1925). As shown already by CLAUS (1889) the trunklimbs of *Nebalia* carry only a superficial resemblance to the true phyllopodia of the Branchipoda. This is shown i.a. by a comparison with *Paranebalia* in which genus the trunk-limbs can be easily traced back to a schizopod pattern of the type found among Mysids. The feeding mechanism and its interaction with the bivalve shell of *Nebalia* in the opinion of CANNON is an adaptation to a life in the muddy habitats frequented by the genus. CANNON concludes (l.c. 369): "From a comparison with *Paranebalia* it is suggested that *Nebalia* evolved from a Mysid, or some other primitive Malacostracan possessing a feeding mechanism similar to that of *Hemimysis* (cf. CANNON and MANTON 1927 and below p. 157), that took to mud-living habits. The foliaceous limbs are in no way primitive but evolved from typical biramous Malacostracan limbs in connection with the new method of filter feeding."

It is against these considerations the arrangement of the nervous system and the main sense organs in the Leptostraca and particulary *Nebalia* must be seen. It would, perhaps, have been a more adequate plan to deal with the Leptostraca

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after the Mysids. However, the Leptostraca actually posses certain primitive features, e.g. the extra abdominal segment, which seems to justify their traditional position as a very primitive group among recent Malacostraca. It seems probable that the second alternative of CANNON is the more plausible and that they were derived, not from Mysids as we know them to-day, but from more primitive Malacostraca, which, however, in many respects and certainly in the structure of the trunk limbs approached the Mysid type.

The nervous system in *Nebalia* forms a straight line, and the brain is not deflected in a dorsal direction. The compound eyes flank the anterior margin of the body proper, only the rostrum, which is a thin outgrowth from the dorsal surface of the body, reaches beyond them. The antennulae are anteroventral and the antennae lateroventral.

The labrum in *Nebalia* is comparatively small, the atrium oris, which is short and fairly wide, points obliquely backwards, and so do the mouth opening and the distal part of the stomodaeum. The anterior part of the ventral surface of the body is not bent upwards as is the rule e.g. among the Peracarida but forms a direct continuation of the posterior parts. Immediately in front of the labrum, however, there is a deep fold, so that the outer wall of the epistome is vertical, i.e. more or less perpendicular to the length axis of the body. In front of the epistome the anterior margin of the fold runs again almost vertically, till it reaches the height of the base of the labrum from where it again bends anteriorly between the bases of the antennulae. We shall presently have reason to revert to the possible significance of this fold.

There is only one slight anomaly in the straight course of the connectives of the central nervous system, but it may possibly have a certain morphological significance. In the maxillary region the ventral nerve chord begins to curve very slightly upwards. This tendency is also to be seen in the posterior part of the circumoesophageal connectives, which, however, soon change to a horizontal course and even slope slightly downwards into the tritocerebrum. This very slight curvature of the nervous system lies in the mandibular region, in fact it could be described as a very low arch over the mandibles (cf. fig. 14). This might be explained by purely mechanical reasons, for the mandibles, projecting into the atrium oris, are fairly bulky and might, figuratively speaking, have pushed the nervous system out of their way in a dorsal direction. Taken in conjunction with other facts, however, the arrangement seems to indicate another, and perhaps more probable explanation. If we accept the mode of feeding occurring in *Nebalia* as a secondary development, as made highly probable by CANNON, there are reasons to believe that *Nebalia* and the Leptostraca in general were derived from Crustacea having the compound eyes frontal or even almost dorsal and the protocerebral part of the brain deflected upwards (cf. below p. 157), these topographical relationships being taken in their functional and not their strictly morphological sense. If this is the case, both the horizontal course

of the nervous system, the arch over the mandibles, the deep fold in the body wall anterior to the labrum, and the ventral position of antennae and antennulae become parts of one coherent picture, for all these morphological features are the natural consequence of a bending downwards of the anterior part of the head. As shown by CANNON (l.c.) the position of the rostrum, the eye-stalks, the antennular and the antennar bases are essential to the peculiar mode of feeding adopted by *Nebalia*.

If the head is bent down as the consequence of a secondary development the arching of the connectives over the mandibles will probably be the result of the nervous system being dragged downwards against the mandibular region, not of the mandibles pushing the nervous system out of their way. Similarly the deep fold of the body wall anterior to the labrum would signify that the inflection of the head took place some distance in front of the labrum. If the Leptostraca were originally filter feeders like the Branchiopoda there is no apparent explanation of this deep fold, nor really of the arching of the nervous system over the mandibles. The large mandibles of the Euphyllopoda produce no similar effect. We shall revert to these questions somewhat later on (p. 171 ff).

Syncarida. A few direct observation of the feeding habits of the Syncarida were published by SMITH (1908), but few details were available until MANTON (1930) made field observations on *Anaspides* and *Paranaspides*. Shortly before that CANNON and MANTON (1929) made a comprehensive study of the arrangement of the mouth appendages and certain other morphological features and could draw some important conclusions on the modes of feeding open to the Syncarida.

The result of all these investigations is that the Syncarida like most other primitive Malacostraca feed according to two methods (cf. p. 157). They posses a filtering mechanism based upon the activity of the "maxilla and adjacent limbs" (CANNON and MANTON 1929 p. 175) and the power of picking up larger food particles by means of the endopodites of some of the trunk limbs and the mandibular palp.

According to the observations of MANTON (1930) it is only *Paranaspides* that makes full use of its filtering apparatus, although it also eats "slime, detritus, and diatoms" covering the weeds in the water (MANTON 1930 p. 793). *Anaspides,* "although possessing a maxillary filtering mechanism, does not filter the water in which it swims as does *Paranaspides,* a Mysid, or a Euphausid" (MANTON l.c. p. 796). Instead it lives on detritus, algae, and various animals such as small tadpoles and worms. It spends most of its time on the bottom, walking or halfswimming, and only swims for longer distances away from the bottom when frightened or when moving from one boulder to another (MANTON l.c.). MANTON also found that it never in a healthy condition swims on its back but always rights itself when it starts swimming if it happens to be crawling upside down among weeds or stones. The eyes are not used for locating food

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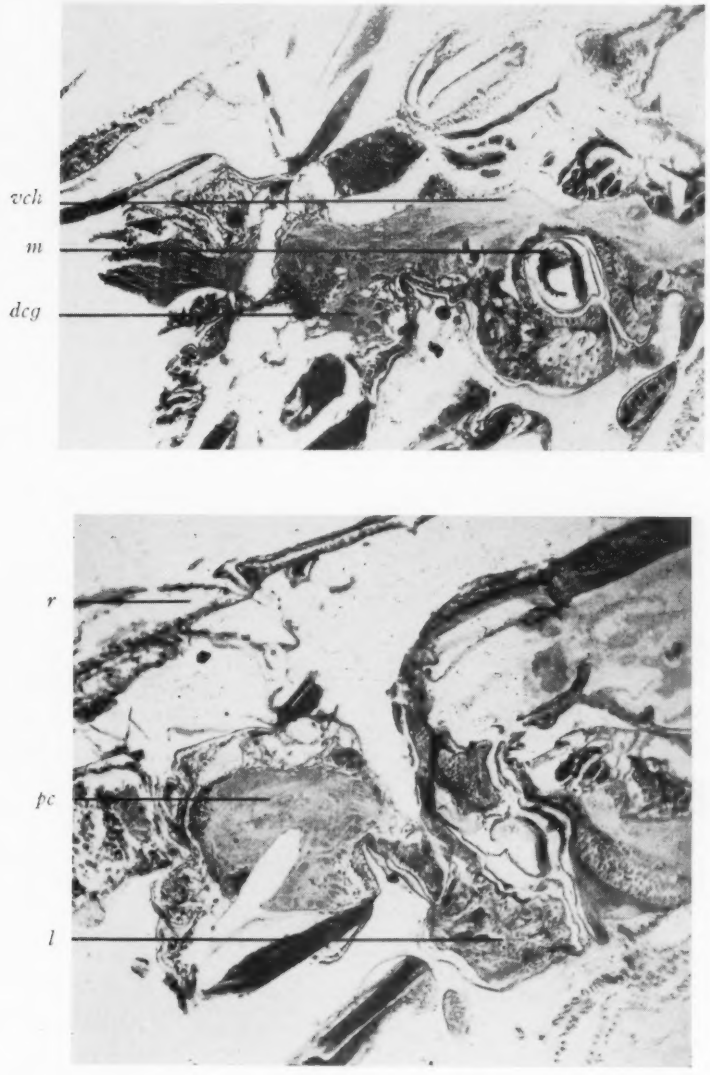

Fig. 14—15. Two parasagittal sections through the head of *Nebalia* cf. *bipes* (from Chile). Note the straight nervous system and the ventral position of the brain.

and face upwards and outwards. On the other hand the tips of the first endopodites are probably sensory. *Anaspides* digs in the bottom with its second to fifth endopodites apparently in search for food.

In *Koonunga* the filtering apparatus has been dispensed with altogether (CANNON and MANTON 1929 pp. 184 ff.). Probably *Koonunga* grips large food particles with the first thoracopod and lifts them to the mouth to be triturated

by the mouth parts. The mouth parts of *Micraspides* according to NICHOLLS (1931) and those of *Bathynella* according to CANNON and MANTON (l.c. p. 186) suggest a feeding mechanism similar to that of *Koonunga*. As far as I know no direct observations of the feeding methods of the last three genera are yet published. In sections of *Micraspides calmani* kindly lent to me by Professor HANSTRÖM, I found the anterior part of the intestine filled in part with fairly large fragments which gave the impression of being plant remains, probably plant detritus.

The topography of the mouth region and the distal part of the stomodaeum in *Paranaspides, Anaspides,* and *Koonunga* can be studied in the excellent diagrammatical illustrations of CANNON and MANTON (l.c.). Personally I studied serial sections of *Micraspides* and *Paranaspides* from professor HANSTRÖM's collection as well as some series of *Anaspides* and also of *Koonunga,* kindly supplied by Dr. F. H. Drummond, Melbourne. Drawings of *Micpraspides* will be found in NICHOLLS and SPARGO (1932) and of *Bathynella* in CHAPPUIS (1927), of *Anaspides* also in SMITH (1909).

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In all genera the distal part of the stomodaeum is perpendicular to the ventral side of the body and comparatively wide, and there are no differences in this respect correlated to the mode of feeding. The atrium oris and labrum, on the other hand, show some most interesting modifications, which are obviously correlated to the feeding method. In the mainly filter-feeding *Paranaspides* the atrium oris is directed somewhat obliquely backwards and covered by the large labrum the length axis of which forms a very acute angle with the length axis of the body. In *Anaspides,* which genus, as noted above, has got a filtering apparatus which it does not use, the labrum is still very long, but its length axis is perpendicular to that of the body so that it hangs down in front of the wide atrium oris, which opens in a ventral direction. In *Koonunga,* finally, the labrum projects but little, and its posterior margin is receding so that the atrium forms a wide funnel with the opening directed ventrally.

The eye-stalks in *Paranaspides* and *Anaspides* are attached close to the anterodorsal margin of the head. The antennulae in all genera are facial in position and the antennae ventrolateral.

The nervous system of *Anaspides tasmaniae* was described in detail by HANSTRÖM (1934). Personally I have examined serial sections of the brain in *Anaspides, Koonunga* and *Paranaspides.* In all genera the axis of the nervous system bends rather sharply in a dorsal direction just in front of the stomodaeum, and the curvature continues all through the brain so that the deutocerebrum forms the part of the brain which lies nearest to the functional anterior end of the body, while the protocerebrum lies above and somewhat behind it (cf. HANSTRÖM l.c. fig. 18 p. 28).

Peracarida. During the discussion of the Leptostraca and the Syncarida the filter feeding method of the Mysids was repeatedly referred to as representing,

in all probability, the basic mode of feeding among the Malacostraca. This was the conclusion drawn by CANNON and MANTON (1927) after their important investigation of *Hemimysis lamornae*. It is not necessary here to give any extensive recapitulation of the method by which *Hemimysis* obtains its food. A food current is produced in two ways, viz. by means of the rotating movements of the maxilla which sucks water into the filter mechanism constituted by the mouth parts and by the thoracic exopodites, which by their swimming movements produce swirls sucking water with suspended particles into the ventral food-grove along which the food is conveyed forwards towards the maxillae. According to CANNON and MANTON (l.c. p. 237) the maxillary apparatus is independent of the other one and may well be much older phylogenetically. As shown by MANTON (1930) *Paranaspides* uses the maxillary apparatus unaided by the thoracic exopodites.

Besides using the filter mechanism *Hemimysis* and probably many Mysids with a similar mode of life (*Praunus* according to DEPDOLLA 1923, *Mysis relicta* according to verbal information from Miss CHARLOTTE HOLMQUIST) pick up large food-masses (e.g. remains of other Mysids) from the bottom by means of the thoracic endopodites and lift them to the mouth parts.

In *Hemimysis lamornae* the atrium oris opens in a nearly caudal direction into the groove between the paragnaths. The mouth opening itself is directed nearly ventrally and from there the distal part of the stomodaeum runs upwards and slightly forwards (CANNON and MANTON l.c. pl. 2). I have studied sections of *Neomysis vulgaris*, *Praunus flexuosus*, and *Mysis relicta* from Miss HOLMQUIST's collection and found essentially the same arrangement. The atrium in all three species opens caudally, the mouth is directed ventrally or more or less obliquely caudally while the ascending stomodaeum is inclined much forwards in *Praunus* and less so in *Mysis* while in *Neomysis* it is short and directed almost straight upwards.

In all the genera referred to above, and on the whole in Mysida in general, the antennae are ventrolateral, the antennulae anteroventral or facial, and the eyes anterodorsal.

The nervous system in *Praunus*, *Neomysis* and *Mysis* forms a straight line past the mouth region and through the tritocerebrum into the deutocerebrum. Only in the transitional area between the deutocerebrum and the protocerebrum is the length axis bent sharply dorsally, so that the protocerebrum lies, on the whole, above the deutocerebrum (fig. 16).

So little seems to be known concerning the mode of feeding of the Tanaidacea (cf. ZIMMER 1927 p. 693) that they were not included in the present investigation.[1] The Isopoda, on the other hand, are comparatively well investigated with

[1] Professor K. LANG kindly called my attention to an important paper by DENNELL: "On the feeding mechanism of Apseudes talpa, etc." (Trans. Roy. Soc. Edinb. vol. 59, 1937—39) which I had previously overlooked. It turns out that *Apseudes* is a filterer, and Professor LANG further informs me that its atrium oris is directed backwards.

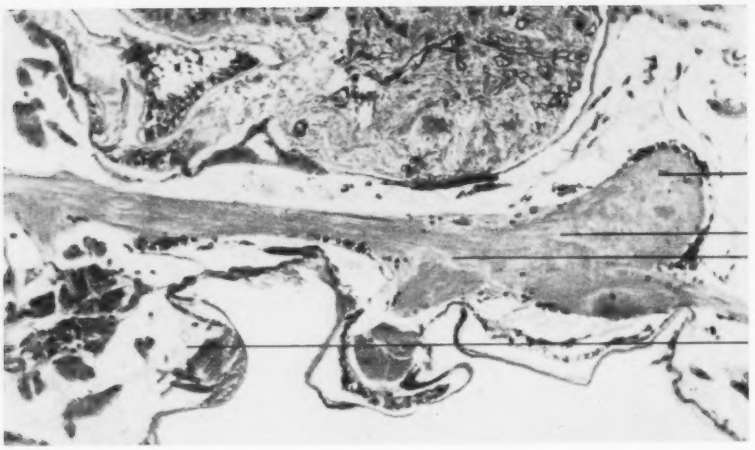

Fig. 16. Parasagittal section through anterior end of nervous system of *Praunus flexuosus*. Note the straight connectives and the ventral position of the brain, where only the protocerebrum is slightly deflected in a dorsal direction.

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respect to the choice of food etc. and display some interesting morphological modifications.

The Flabellifera are predators and scavengers and even facultative ectoparasites, making use of their strong biting mouthparts.

Great numbers of Isopods, both aquatic and terrestrial, are herbivorous, gnawing plants and plant remains with their powerful mouthparts.

Instances of this mode of feeding to be dealt with below are *Asellus, Idothea,* and *Oniscus. Munnopsis typica* was seen by HULT (1941 p. 131) to pick up food particles from the bottom with the two anterior pairs of pereiopoda and lift them to the mouth opening. In the survey given by ZIMMER (1927 p. 738) the strongly flattened genus *Serolis* living on sand and other soft bottoms is said to eat detritus and to be possible predatory (cf. below p. 160).

The Flabellifera are particularly interesting because of the various primitive morphological features retained among them, e.g. in the structure of the mouthparts and the abdomen. The oesophagus in *Eurydice pulchra* points obliquely forwards-downwards, the labrum occupies the anteroventral corner of the body, and in front of it the ventral surface of the body is bent upwards. As will be seen in the next few paragraphs this tendency has become even more pronounced in various other sub-orders.

In *Asellus aquaticus, Idothea granulosa,* and *Oniscus asellus* the oesophageal part of the stomodaeum is directed obliquely forwards rather than ventrally. This has gone so far that the tip or anterior margin of the labrum acutally forms the functional anterior end of the body. The same arrangement, perhaps even in more extreme form is found in *Munnopsis typica* (figs. 17—18). In the types

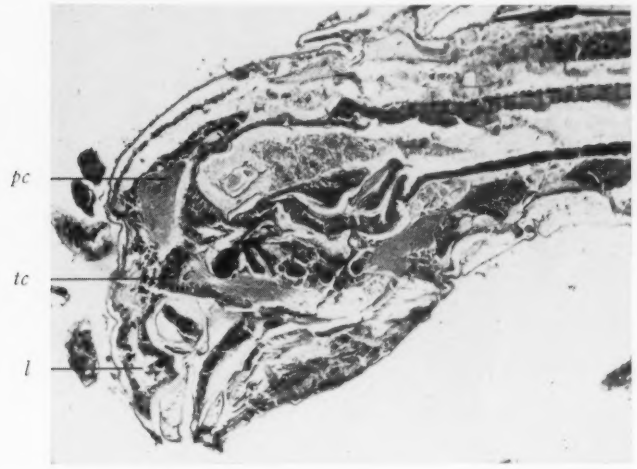

Fig. 17. Parasagittal section through anterior end of *Asellus aquaticus*. Note the sharp dorsal curving of the brain, the anterior end of which tips over backwards, and the antero-ventral position of mouth and atrium oris.

referred to here we find at last that the anterior margin of the labrum and the clypeus become "facial or dorsal" as believed by SNODGRASS (1951 p. 107) to be the original arrangement among mandibulate arthropods in general.

In all the types of Isopods mentioned here the deflection upwards and even backwards of the anterior end of the body has removed the antennae and antennulae from the functional ventral side of the body. The sessile compound eyes are frontolateral or more often dorsolateral.

The dorsal deflection of the body is very clearly reflected in the topography of the brain. In *Eurydice,* where the antennae and antennulae are facial, with the bases of the antennulae placed very near the (functionally) anterodorsal margin of the head, and the eyes are dorsolateral, the length axis of the brain is vertical, roughly perpendicular to the length axis of the body. Thus in *Eurydice* the (morphologically) anterior part of the brain is not tilted over backwards. Such a tilting over backwards is, however, to be noticed in *Idothea,* where the pushing forwards of the mouth has proceeded somewhat further than in *Eurydice,* and it is very pronounced in *Asellus* (fig. 17) where the distal end of the labrum lies a good deal in front of the bases of the antennae. Among the genera investigated this tendency culminates in *Munnopsis* (fig. 18), where the length axis of the brain is bent over so far backwards that it is not very far from parallel with that of the ventral chain, although it runs in the opposite direction. I had no opportunity to investigate the inner morphology of any Gnathiidae with their large, protruding mandibles and receding "fore-head". In all the genera mentioned above, with the exception of *Eurydice,* the trito-

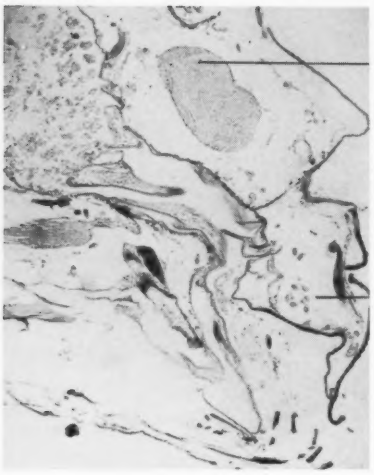

Fig. 18. Sagittal section through the anterior end of *Munnopsis typica*, which, like the majority of the Isopoda shows a topography of the head closely similar to that of *Asellus*.

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cerebrum constitutes the part of the central nervous system which is pushed furthest forwards towards the functional anterior end of the body.

Serolis (fig. 19), adapted to a very specialized mode of life, shows some variations within the same general pattern. In the Seroliidae we find the dorso-ventral flattening of the Isopoda in its most extreme form, the body is nearly circular, and when the isopod lies on the sand or soft bottom on which it lives the body forms a low dome, the apex of which is hardly raised above the surrounding bottom, and the coxal plates touch the surface of the latter. Although the mode of feeding was never observed in detail it is obvious that *Serolis* gets its food from the bottom below it. In the intestine of my sectioned specimens I could identify remnants of crustacea, small fragments of mollusc shells, shells of foraminifera, sand-grains, diatoms, and amorphous detritus. The mouth and atrium open in a ventral direction and obviously the mouth parts pick up and sort out food particles from the bottom. The sorting must be fairly effective, for the mineral grains in the intestine are few.

Despite the fact that the mouth lies on the functional ventral side a good deal behind its anteroventral corner, which is formed by the anterior end of the long flat labrum, the oesophagus as in the other types of Isopoda dealt with here runs from the mouth opening obliquely upwards-backwards to the stomach.

In front of the labrum the ventral surface of the head bends abruptly upwards, so that the large antennae become functionally facial and the antennulae antero-dorsal. The eyes are dorsolateral and lie a good deal behind the antero-dorsal margin of the head.

In the central nervous system the circumoesophageal connectives form a straight continuation forwards of the ventral nerve chain and enter the trito-cerebrum without any previous deflection upwards (fig. 19). Actually the

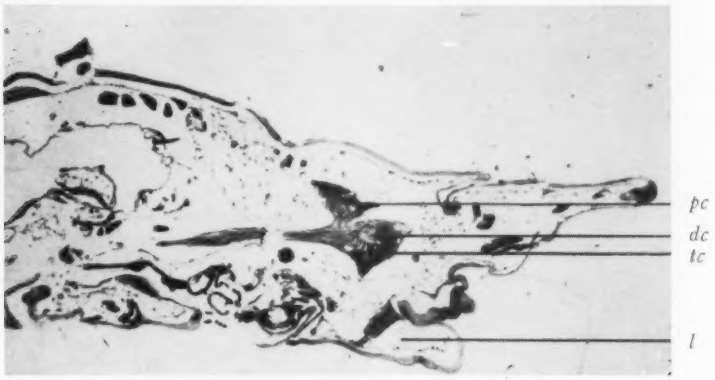

Fig. 19. *Serolis* sp., an abberrant Isopod type.

larger part of the tritocerebrum bulges ventrally and assumes a position antero-ventral to the point where the connectives enter. The deutocerebrum, however, lies on the (functionally) dorsal side of the tritocerebrum, and the area where the upper roots of the antennular nerves leave it is the part of the brain which lies nearest to the (functional) anterior end of the body. The protocerebrum is bent over backwards and lies dorsal to and, on the whole, a good deal behind the deutocerebrum.

In comparison with the Mysids the Isopods, as shown by the discussion on the last few pages, show a number of transformations in the topography of the head region which are, undoubtedly, to be regarded as adaptational changes of the original malacostracan pattern. The Isopods have dispensed with filter-feeding, and in connection with their feeding by means of their strong, biting mouthparts the mouth has been pushed forwards. This obiously has taken place at a comparatively early stage in their phylogenetical development, for with comparatively small variations the same Isopod pattern is found in all recent types investigated. We shall revert later to the effect of these topographical changes upon the nervous system (p. 174).

The Amphipoda like the Isopoda show a number of variations with respect to their choice of food. Many of them are scavengers living mostly from dead plants or animals which are chewed by their powerful mouth-parts. The well-known genus *Gammarus* is a case to point. It feeds on all kinds of plant and animal tissue soft enough to be dealt with by its mouth-parts, and judging from the contents of the oesophagus and stomach of sectioned specimens, it also consumes a good deal of detritus and diatoms. BLEGVAD (1921 p. 5) found that if opened but living specimens of *Mytilus edulis* were placed in an aquarium with numerous specimens of *Gammarus* they were soon devoured so that only shells and byssus threads remained.

The feeding habits of the supralittoral Talitrids *Orechestia gammarellus* and

O. platensis were studied by BACKLUND (1945 p. 117) who found that they "eat all dead organic matter, both animal and vegetable". They gnaw the softer parts of *Zostera* and *Fucus* thrown up on the beach and cannot be kept in papier maché boxes or cloth bags.

But there also exist other feeding methods among the amphipods. Those of the family Ampeliscidae are particularly interesting, and were described by ENEQUIST (1949) in his extensive discussion of the biology of the soft-bottom amphipods of the Skagerak. Most ampeliscids feed lying on their backs in the opening of their vertical tubes. As an instance we shall choose the group consisting of *Ampelisca brevicornis* and *A. tenuicornis*, the latter of which I have studied morphologically. The two species mentioned and some others, while lying in their tubes as described above, scrape the surface of the bottom round the tube with the antennae which are then bent in over the gnathopods and mouth-parts so that any detritus particles adhering to them can be scraped off and conveyed forwards to the mouth. The species mentioned also feed, although according to ENEQUIST not to the same extent, on suspended detritus which is caught by the current produced by the pleopods. This current passes from the anterior end of the animal over the ventral side of the head and backwards. Food particles are strained off by the mouth parts and gnathopods (cf. HUNT 1925 p. 574) and brought to the mouth opening.

The topography of the atrium and oesophagus are correlated to these various modes of feeding. In *Ampelisca* which is at least partly filter feeding and where in any case the food particles are caught by the postoral appendages and carried forwards to the mouth, the oesophagus runs upwards-forwards from the mouth, but the atrium oris opens in a ventral direction behind a comparatively large and projecting labrum. In *Gammarus* there is a somewhat similar arrangement with an oesophagus running in an obliquely caudal direction from the stomach to the mouth which opens more or less ventrally. *Gammarus* in picking up its food uses the maxillipeds a good deal, and the existing arrangement with a (functionally) ventral mouth opening is apparently well suited to this mode of feeding. *Orchestia* differs considerably from this pattern. *Orchestia* is adapted to feeding on land and on comparatively hard and tough food. The mandibles are the main operators—the maxillipeds of the family Talitridae are comparatively poorly developed—and the mouth opening is directed obliquely forwards which brings the mandibles into a very favourable position for gnawing on the plant remains constituting the main source of food. The atrium oris is very small and almost completely filled by the mandibles, and it forms a direct continuation of the oesophagus which runs forwards-downwards from the stomach. The position of the mouth at the anteroventral corner of the body makes the morphologically ventral side of the labrum the functional anterior end of the body. Thus conditions in *Orchestia* very much resemble those found in various Isopods (cf. p. 158).

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Fig. 20. Parasagittal section of *Ampelisca tenuicornis* showing the straight connectives and the ventral position of the tritocerebrum.

The topography of the central nervous system of the three genera dealt with above show an interesting correlation to the position of the mouth, and the same applies to the eyes and head appendages.

In *Ampelisca tenuicornis* the length axis of the body is not bent upwards in front of the mouth, the compound eyes are antero-ventral and both antennulae and antennae belong to the functional ventral side. In some other species of *Ampelisca* the antennulae are facial. The length axis of the nervous system, however, is not quite straight, the protocerebrum is deflected upwards so that it lies above and somewhat in front of the deutocerebrum (fig. 20). This supports the view — probable in itself — that the straight length axis of *Ampelisca* is secondary and that the genus was derived from ancestors with a cephalic topography of the normal malacostracan type, i.e. with more or less facial antennulae and antennae and with the eyes (when present) dorsal or dorsolateral, as is actually the case in at least two other genera of the same family, *Haploops* and *Byblisoides*. The bending upwards of the axis of the central nervous system which has been partly but not entirely obliterated favours this view. There is also a deep fold in the ventral body wall in front of the epistome, which, as in *Nebalia,* may possibly be an indication of a secondary bending of the head. As already mentioned, *Ampelisca teniucornis* like other investigated species of the genus feeds lying on its back in the mouth of its tube, and the secondary deflection of the head, which brings the mouth into a favourable position to the mode of feeding and turns the cephalic sense organs towards the surrounding water, is a highly probable adaptation to such a mode of life. It is true that *Haploops tubicola* assumes a similar position when feeding (Enequist l.c. fig. 63 p. 351) without having its head bent downwards

Fig. 21. Sagittal section through brain and fore-gut of *Gammarus zaddachi*.

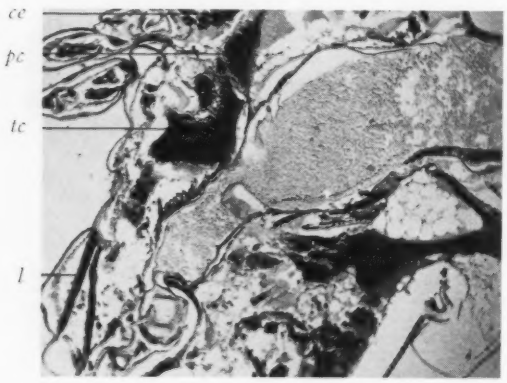

Fig. 22. Sagittal section through head region of *Orchestia platensis*.

as *Ampelisca*, but on the whole the morphology of *Ampelisca* seems to be much more highly specialized than that of *Haploops*.

In *Gammarus* where the mouth opening is also ventral, the ventral side of the body is bent upwards from the anterior margin of the labrum. Both antennae and antennulae are facial, the antennae in an antero-ventral, the antennulae in an antero-dorsal position. The eyes are lateral and extended in a (functionally) dorsoventral direction. As could be expected from this arrangement the axis of the nervous system is deflected rather abruptly upwards in the

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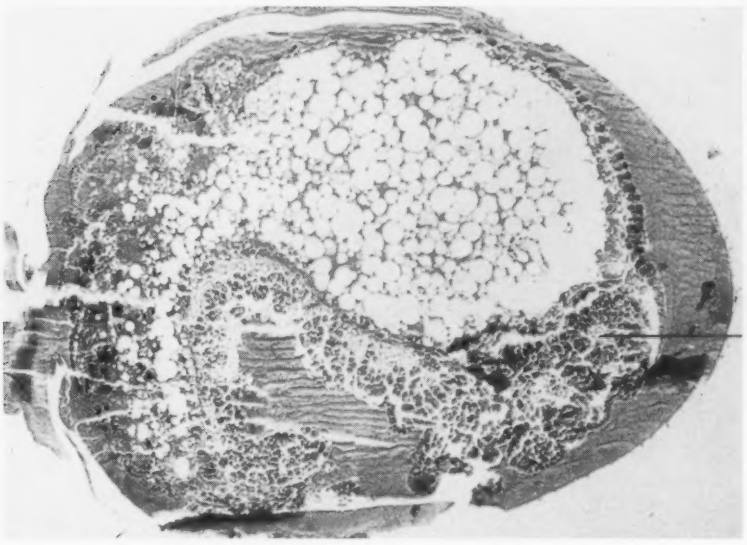

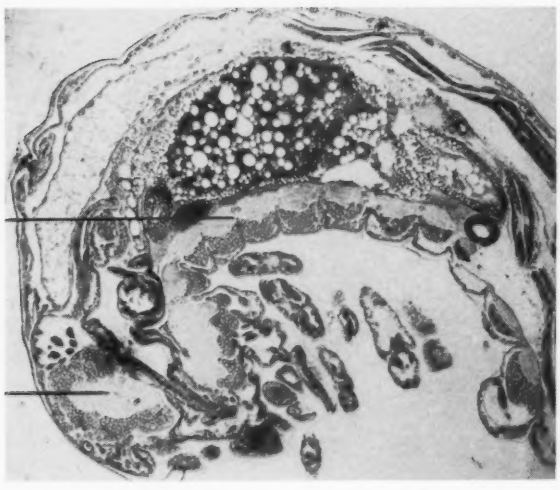

Fig. 23—24. Sections through embryos of *Orchestia platensis*. Fig. 29 (above) comparatively young stage with small limb rudiments, fig. 30 late embryo just before hatching.

posterior part of the tritocerebrum. There is no bending over backwards, however, and the protocerebrum lies straight above the deutocerebrum (fig. 21).

In *Orchestia* the position of the mouth at the (functionally) anteroventral corner of the body makes the whole (morphologically) ventral side of the labrum functionally facial in position. Thus the more or less vertical "face" of

Orchestia becomes very long and both antennae and antennulae become more or less anterodorsal in position. The eyes are dorsolateral. The nervous system is bent upwards very sharply in the mandibular-tritocerebral area, and in front of the bend the axis of the brain runs more or less parallel to that of the foregut although in an opposite direction. The anterior part of the brain is bent over backwards so that the protocerebrum lies above and behind the deutocerebrum (fig. 22).

Already before hatching the topography of the nervous system in *Orchestia* is, on the whole, the same as in the adult. In younger embryos, however, the dorsal deflection is much less pronounced and could be better described as a gentle curving. The protocerebrum although somewhat more dorsal in position than the deutocerebrum is by then clearly the anterior end of the nervous system and shows no tilting over backwards (figs. 23—24).

The topography of the head in the adult *Orchestia* very much resembles that found in most ot the Isopoda dealt with on pp. 158—60. Otherwise the topography of the head in the Amphipoda deviates less than that of the Isopods from the primitive Malacostracan pattern found e.g. in the Mysidacea.

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FORSMAN (1938 p. 65) observed the mode of feeding in the Cumacean *Diastylis r. rathkei*. Further observations on the feeding of Cumacea will be found in papers by ZIMMER (1933), DENNEL (1934), and FOXON (1936).

Diastylis rathkei, when feeding, is dug in as shown in FORSMAN's fig. 5 c (l.c. p. 65). It lies partly on its back, so that the anterior part of the ventral side is directed upwards. As is generally the case with Cumacea the anterior appendages and particularly the maxillipeds are directed forwards. *Diastylis* collects detritus with the aid of the first periopods which scoop up bottom deposit from the area within their reach and bring it to the third maxillipeds which apparently sort it and bring the edible particles to the mouth parts proper.

From the stomach the stomodaeum, mouth, and atrium oris in *Diastylis* form a straight line directed ventrally and somewhat forwards. The mouth lies at the (functional) anteroventral corner of the head, in front of it the ventral body wall bends upwards, so that antennae and antennulae are more or less facial.

The axis of the nervous system is bent rather sharply upwards in the maxillary region and the connectives pass rather steeply upwards to the brain, the terminal part of which is not tilted over backwards. Thus in *Diastylis* the nervous system does not follow the contour of the ventral side nearly as closely as in most Crustacea, but it makes a "short cut" through the posterior part of the head region to the brain proper (fig. 25).

Eucarida. The mode of feeding of the Euphausiacea was never studied in great detail. LEBOUR (1924 p. 405) makes a brief statement concerning *Nyctiphanes couchii*. "The food is brought to the thoracic limbs by a current from behind, set up by the movement of the abdominal limbs, the thoracic limbs forming a sort of basket-like receptacle for the accumulated food. Although the

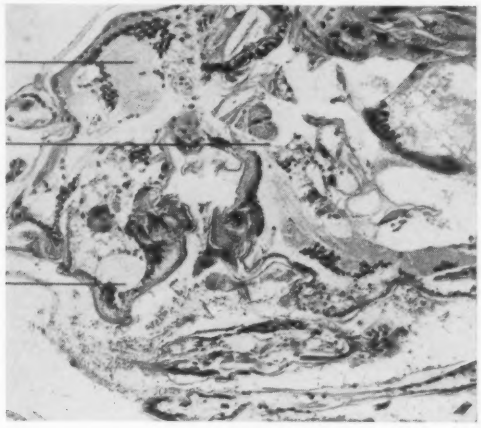

Fig. 25. Parasagittal section through head of *Diastylis rathkei*.

food organisms found inside them were usually unicellular, they sometimes feed on Sagitta and Crustacea." Some general considerations based on the study of appendages but not on the activity of living specimens were contributed by ZIMMER (1913) and CHUN (1896). It seems probable that the majority of Euphausids are at least mainly filter feeders, though some genera, notably *Stylocheiron* and *Nematoscelis* are probably rapacious.

A very good description of the general topography of gut and nervous system in *Stylocheiron* will be found in CHUN (l.c., particularly elucidating is pl. 11 fig. 9), and valuable information on *Euphausia superba* is to be found in ZIMMER (l.c.). HANSTRÖM (1933) described the finer structure of the brain in various genera. He kindly lent me his collection of sections which also permit a survey of the general topography of brain and foregut in the genera *Meganyctiphanes, Thysanoessa, Nematoscelis,* and *Stylocheiron.* The description by CHUN (l.c.) which was referred to above, turns out to be applicable in its essentials to all the genera dealt with. From the stomach the oesophagus runs obliquely downwards-backwards to the mouth, which is situated on the functional ventral side, well behind its anterior corner. The antennae are ventral and the antennulae anteroventral. In front of the antennulae the ventral side is bent upwards, though not very abruptly, and the bases of the eye-stalks are facial. The length axis of the central nervous system is hardly bent upwards until in the deutocerebrum, and even then the bending is not abrupt. The protocerebrum in this way comes to lie in an antero-dorsal position and is the topographically anterior part of the nervous system.

Most Decapoda are rapacious or scavengers living from plant or animal tissue. Other modes of feeding also occur, thus e.g. the burrowing Thalassinidea, *Callianassa* and allied genera, are substratum feeders in the wider sense. They do not eat the substratum indiscriminately as many annelids do, but with

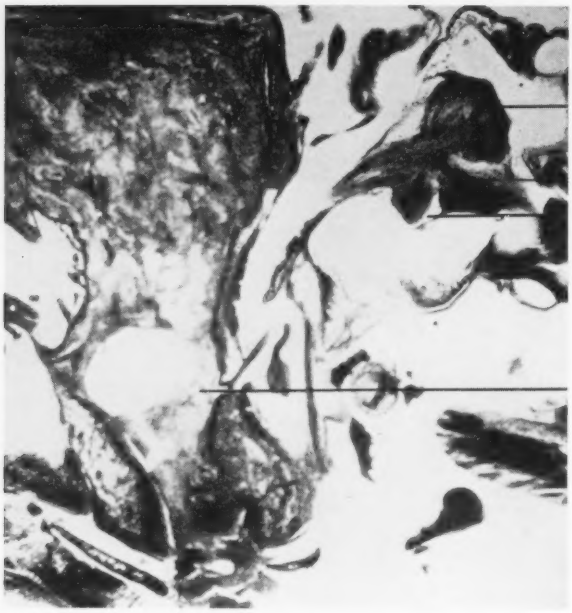

Fig. 26. Parasagittal section through head of *Pandalus montagui*.

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their mouth-parts they sort out edible particles from it (G. E. and N. Mac Ginitie 1949). Anomuran crabs of the genus *Emerita* strain food particles from the water by means of their antennae (Jones 1936). Comprehensive studies of the working of the mouth-parts of Palaemonid prawns and of *Carcinus maenas* were published by Borradaile (1917 and 1922), a comparatively recent survey of the structure of decapod mouth-parts with regard to functional aspects was made by Balss (1940). I have studied the general topography of the anterior end of various species, especially *Homarus vulgaris, Pandalus montagui, Callianassa uncinata,* and *Hyas coarctatus.*

Many Decapods are highly specialized to various modes of life, a well-known fact reflected in the extreme degree of variation of their general appearance. As a rule, however, the mouth opening is situated on the ventral side well behind the anterior end. In the burrowing Thalassinides, however, it lies very near the functional anterior end, but it is still definitely ventral. This would seem to be a more or less necessary adaptation for a substratum feeder in burrows. In connection with this dislocation forwards the oesophagus of *Calianassa* has come to form an angle of more or less than 45° upon the length axis of the body, ascending obliquely upwards-backwards from the mouth to the stomach. Otherwise the oesophagus mostly runs straight dorsally from the mouth up to the stomach.

Fig. 27. Parasagittal section through head of *Hyas coarctatus*. Note the very long connectives.

The eyes, antennulae and antennae generally lie very close together at the functional anterior end of the body, and often they lie very nearly in the same horizontal plane. As is generally the rule in bulky animals the nervous system takes up a comparatively much smaller part of the interior of the body than is the case in small forms. The brain lies at the anterior end of the body and generally shows a deflection upwards somewhere in the transition area between tritocerebrum and deutocerebrum. As a rule, however, the length axis of the anterior part of the brain still slopes a little forwards so that the protocerebrum lies somewhat in front of the deutocerebrum. The oesophageal connectives of the Decapoda are very long and generally run horizontally or with a slight inclination upwards. For general information of the topography of the nervous system of Decapoda cf. surveys by HANSTRÖM (1928) and BALSS (1941 and 1944).

If thus the topography of the adult Decapods shows some modifications which appear rather extreme when compared with the normal crustacean pattern, it is interesting to note that these modifications appear fairly late from an ontogenetical point of view. Figs. 28—30 show a late metazoea stage of *Leander squilla*, and it will be evident at once that the topography of its anterior end shows a very close resemblance to that found in Mysids and Euphausiacea. As could be expected in this small animal, the nervous system is comparatively voluminous. The circumoesophageal connectives are short, and the deflection upwards of the axis of the central nervous system takes place in the deutocerebrum. The antennae are definitely ventral and the antennulae anteroventral, just as we found them among the Euphausiacea.

Stomatopoda (Hoplocarida). In many respects the Stomatopoda represent the most modified of all the Malacostracan types. They are all rapacious, catching the prey with the second maxilliped and bringing it to the maxillar region by means of the 3rd to 5th maxillipeds which also tear it to pieces (cf. Balss 1938 pp. 109 ff.). The mouth lies on the ventral side far behind the anterior end. The atrium oris opens in a ventral direction behind the very long and flat labrum, the oesophagus runs forwards and upwards into the complicated stomach which also in many respects differs from that of other Malacostraca (cf. Balss l.c. pp. 33 ff.).

Thanks to hinges in the external skeleton of the head region the anterior part, carrying the eyes and the antennulae, can be moved freely and thus assume varying positions relative to the length axis of the rest of the body. The circumoesophageal connectives are very long and the brain lies in the anterior part of the head. The length axis of the brain is parallel to that of the movable anterior part of the head ("Vorderkopf" of German writers, protocephalon of Snodgrass 1951). The structure of the brain in *Squilla mantis* was described by Hanström (1931). Unfortunately the nervous system of Stomatopod larvae was never investigated and I had access to no material suitable for such a purpose.

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g. GENERAL REMARKS

In the previous sections of this chapter representatives of nearly all major Crustacean groups were surveyed and their mode of feeding, sometimes their general mode of life, and the topography of their foregut and brain were considered. There appear to be some obvious correlations, which may be somewhat difficult to trace in the highly specialized forms, such as crabs and stomatopods, but which stand out clearly in the case of more primitive or at least less complicated groups.

The position and orientation of mouth opening and stomodaeum appear to be adaptable and closely correlated to the mode of feeding, and two particular modes of feeding, filtering and browsing or gnawing appear to have always an equally marked though opposite effect on the topography of the oral area. With the exception of the Ostracoda, filterers, notwithstanding the method by which the actual feeding currents are produced, always bring the food from behind to appendages in the immediate neighbourhood of the mouth. These appendages, particularly the maxillae, maxillulae, and mandibles, after dealing with the food particles in various ways finally bring them into the mouth opening proper, generally aided by the secretion of the labral glands. Throughout the Crustacean system, wherever true filterers are to be found, i.e. among the Branchiopoda, Mystacocarida (probably), Copepoda, Leptostraca, Syncarida, and Peracarida, it could be shown that the mouth opening is ventral and opens more or less backwards. When the last is not true of the mouth opening itself

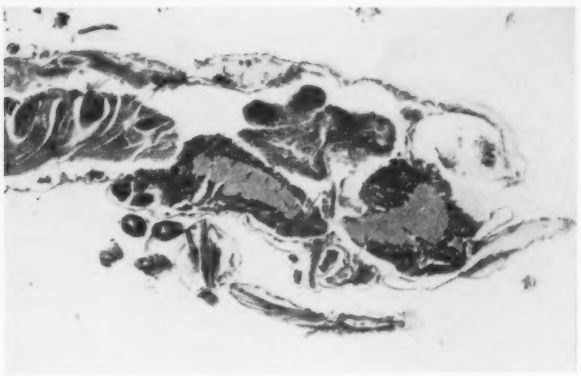

Fig. 28—30. Three sections through head of metazoea of *Leander squilla*. The nervous system is much more concentrated than in the adult, the antennae are ventral and the antennulae anteroventral.

it applies instead to the atrium oris. The labrum is large or fairly large, and its length axis is directed more or less obliquely backwards.

Conversely in Crustacea feeding by means of browsing and gnawing, i.e. by nearing the mouth itself to the source of food which is then attacked by the mouth parts proper, the mouth is directed ventrally or even forwards. Simultaneously the ventral side of the body in front of the mouth is generally deflected in a dorsal direction. Functionally this meets the need of bringing the ventral body wall and the basal parts of the pre-oral appendages out of the way, so that the mouth parts are not hampered when dealing with a source of food in front of the crustacean. As shown by the previous sections of this chapter such an arrangement developed independently in various groups, in Harpacticoida, to a certain extent in the Ostracoda, further in the Isopoda, and in the terrestrial Amphipoda.

In forms which bring their food to the mouth by means of the thoracic appendages the position and direction of the mouth opening and atrium is more variable, as could be expected.

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It is very instructive to study certain groups where different modes of feeding are practised by animals, the general morphology of which is closely similar. In the discussion of the various orders several instances of such variation within the same group were encountered. Thus among the Copepoda the Calanoidea are mainly filterers and have the mouth and atrium opening directed backwards, behind a fairly large labrum. In the rapacious *Cyclops* the mouth and atrium opens in a ventral direction and lies comparatively far forwards, though still on the functionally ventral side. Finally, in the browsing Harpacticoids the mouth lies at the (functional) anterior corner of the ventral side, and the stomodaeum is directed obliquely forwards.

An exactly analogous series can be found within a group from a quite different part of the crustacean system, the Amphipoda. *Ampelisca* is at least partly a filter feeder and its atrium oris opens obliquely backwards. *Gammarus* is omnivorous and uses its maxillipeds to a fairly large extent in order to bring the food to the mouth. Its mouth opening is definitely ventral and its atrium oris opens in a ventral direction. *Orchestia* gnaws fronds af dead algae, its mouth and atrium are directed obliquely forwards and are situated at the functional anteroventral corner of the body.

A somewhat similar series also exists among the Syncarida, with *Paranaspides* as a partial filter feeder with the atrium opening backwards, while *Anaspides* and *Koonunga* scrape up food particles by means of their thoracopods. In both the latter genera the atrium opens in a ventral direction, but *Koonunga* with its semi-terrestrial habits and with appendages much less primitive than those of *Anaspides* also has a much smaller labrum and thus a wider entrance to the mouth than the other. Undoubtedly a closer scrutiny of various groups would reveal the existence of more instances of the same tendency. The instances

quoted, however, should be sufficient to establish the general validity of the suggestion made above concerning the adaptability of the topography of the oral region relative to the mode of feeding.

Concerning the phylogenetic significance of the various modes of feeding all comparatively recent writers on the problem, CANNON and MANTON (several papers, especially 1927), STORCH (several papers) and ERIKSSON (1934), despite diverging opinions on various important details, agree upon the filter feeding as the oldest feeding method of the Crustacea. As already pointed out (pp. 133 ff.) it seems, indeed, probable that feeding by means of currents produced by the appendages was one of the fundamental achievements necessary to release the selective forces which brought about arthropod development and differentiation.

It is not surprising, therefore, to find feeding by means of currents produced by some of the appendages, as well as a posteriorly directed atrium oris, in all the oldest and most primitive groups of Crustacea, in the Euphyllopoda, including the Devonian *Lepidocaris,* in the Mystacocarida, which have certainly not undergone any significant changes for hundreds of millions of years, in the Calanoida, most primitive of the recent Copepod groups, in the Mysidacea, which certainly represent a primitive and central type from which other Malacostraca can be derived (cf. i.a. SIEWING 1951), in *Paranaspides,* the most primitive surviving genus of the Syncarida, which have not changed very much since the Carboniferous, in the Leptostraca, which, although they were probably derived from forms resembling the Mysids (cf. p. 152), yet go back to the earlier half of the paleozoic epoch. The Ostracoda, too, partly rely upon feeding currents, although their mechanism is a very different one from the groups mentioned above. Apparently they branched off very early from the main crustacean stock, for their shells are among the oldest recognizable crustacean remains, but very little is known at present about the chain of adaptations by which they attained their highly aberrant but also highly succesful and very uniform structural plan, which, despite its great age, is certainly far removed from the ground plan of the most primitive Crustacea.

On the other hand there is every reason to believe that the great number of other phylogenetically old filter feeders referred to in the preceding paragraph retain between them a great number of morphological traits which are truly primitive. Within the various major groups it is often possible to trace the adaptational changes of types with a different mode of life back to a morphological pattern most closely resembling that of the filter feeders within the respective group. Instances illustrating this statement are to be found in the descriptions of the topography of Branchiopoda, Copepoda, Peracarida, and Syncarida in the present and previous sections of this chapter. It should perhaps be pointed out that the comparison between various amphipods on p. 163 is not a case to point for the filter feeding in Ampeliscids is certainly a secondary acquisition. However, there exist very strong reasons to believe that within

the respective taxonomical groups the morphological pattern of original filter feeders is the one which, among the recent representatives of the group in question, most closely resembles the original structural plan. Keeping in mind the dangers always inherent in generalizations of this kind and with due reservation for exceptions from the rule, we may extend this statement to cover also the structure of organ systems not directly involved in the feeding mechanism, as long as they are not specialized to meet functional demands from a mode of life which, in other respects, differs from the one of the primitive forms. In practice it will, of course, often be very difficult to decide whether any such secondary modification has taken place, although in some ceases it is obvious (e.g. reduction of eyes in many bathypelagic crustaceans, confluence of paired compound eyes in Conchostraca and Cladocera).

As already pointed out (p. 170) changes in the mode of feeding bring about very considerable changes in the topography of the oral area. The next step, then, will be to trace the influence of these changes upon other organ systems, especially the central nervous system, and, on the whole, on the topography of the anterior end of the body.

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Ontogenetically the crustacean mouth opening is formed on the ventral side, the appendages of each side up to and including the antennulae are arranged in an unbroken series clearly pertaining to the ventral side, and the same applies to the nerve chain, which is straight or, rather, closely follows the course of the ventral body wall from which it was derived. This arrangement of limb and ganglion rudiments will be found until fairly late in the embryological or even larval development also of species which as adults show considerable deviations from it.

If, as was done in previous sections of this chapter, we examine a number of filter feeders where it is practically certain that filter feeding is original and not due to any secondary development (e.g. the Euphyllopoda, the Mystacocarida, the Calanoida, and the Mysidacea) we shall find that the arrangement of the appendages and the central nervous system very closely approaches the general plan found ontogenetically. In all these groups the antennae are ventrolateral, the antennulae likewise ventrolateral or more often anteroventral and the axis of the central nervous system forms a more or less straight line through the tritocerebrum into the deutocerebrum. The notable exception with respect to the nervous system is the Notostraca where the brain is deflected sharply upwards, the compound eyes and the nauplius eye being dorsal, while the ventral antennulae and antennae are very much reduced in importance. As was explained previously (p. 142) there are good reasons to believe that this is an adaptational response to certain aspects of the mode of life of the group.

Provided that the assumption made above concerning the structural primitivity of the original filter feeders is correct, and considering the fundamental agreement with the pattern recognized as ontogenetically primary, it seems very highly

probable that the pattern described above with ventral mouth, ventral cephalic appendages and straight nervous system represents the fundamental structural plan of the Crustacea.

In forms where filter feeding has been suspended and where mouth and atrium open straight ventrally or more or less obliquely forwards (cf. p. 172) the topography of the area in front of the mouth becomes profoundly affected. However, it should be pointed out here that considerable topographical changes in the same area may also be due to other causes. If, in order to simplify the argumentation, I may express myself somewhat teleologically, there are above all two types of topographical change in the preoral area meeting new functional demands, viz.:

1) a lifting of the ventral surface of the body anterior to the mouth in order to prevent its hampering the mouth parts in dealing with food lying on the ground or bottom in front of the animal.

2) a dislocation upwards of the main sensory centres, eyes, frontal organs, and antennulae, in order to deal more efficiently with stimuli emanating from the area in front of and above the animal.

It should be pointed out a once that the changes produced by a functional demand such as suggested in p. 1 could equally well be produced by an actual pushing forwards of the mouth past the brain and the bases of antennae and antennulae. Possibly both these processes interact and contribute to the final result (cf. p. 186). Functional demands of the type mentioned in p. 2 will arise most easily when a pelagic form adapts itself to a benthic mode of life. But they may also be brought into existence when a change of diet takes place, e.g. when a filter feeder is transformed into a rapacious form, which instead of feeding more or less indiscriminately must single out and attack individual prey. Thus there may be a close interaction between various different influences, and the two alternatives set down above should not be understood to be antagonistic or even necessarily independent of each other but merely to represent two main trends along which selection could act.

The topographical changes implied in the lifting of the anteroventral surface of the body from the ground (respectively the dislocation forwards of the mouth) are mainly the following ones. If the case is not an extreme one the anteroventral corner of the labrum will also represent the functional anteroventral corner of the body, the antennae will lie more or less directly below the antennulae and the compound eyes above or somewhat in front of the antennular bases. In the nervous system the main deflection upwards of the main axis will lie in the transition area between tritocerebrum and deutocerebrum, but generally the protocerebrum will be somewhat anterior to the deutocerebrum or will lie more or less straight above it. This is a pattern found in a very great number of Crustacea dealt with previously in this chapter, e.g. the Harpacticoida, most aquatic Amphipoda, the Syncarida, the Euphausiacea.

In the most extreme cases on the other hand, as represented among the Crustacea in the first place by many Isopods and terrestrial Amphipods, probably also by some Tanaids, the distal (originally posterior and ventral) end of the labrum becomes the functional anterior end of the body, and the entire ventral margin of the labrum becomes facial and somewhat dorsal. The antennae are inserted sometimes slightly in front of the antennulae, sometimes below them, sometimes they are placed laterally. The compound eyes on the other hand are in these forms always placed well behind the antennar and antennular bases. The length axis of the brain is bent upwards, generally somewhere in the tritocerebral region, and in the morphologically anterior part of the brain it even tips over backwards so that the protocerebrum comes to lie behind the deutocerebrum. The actual anterior point of the nervous system is not always the same but often lies where the antennar or antennular nerves leave the brain.

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It is worth noting that the topographical changes produced in the manner described above generally give a very consistent picture which, in principle, varies but little from group to group. Where variations of the pattern occur they befall, in the first place, the eyes, the compound as well as simple ones, for they are very susceptible to adaptational changes brought about in the manner implied by pt. 2 on p. 175.

Life in a terrestrial habitat seems to favour an anterior position of the mouth and the jaw apparatus. We met with a few instances of this tendency among the Crustacea, particularly among the terrestrial Isopods and Amphipods. But few groups of Crustacea have representatives on dry land. However, a comparison with terrestrial mandibulate arthropods shows the same tendency in a still more marked degree than was the case among the Crustacea, for both among the Chilopoda and the Insecta the mouth is, in a very great number of cases, placed very far forwards. The position of the single pair of antennae and of the eyes as well as the arrangement of the nervous system then very closely follows the pattern described in the case of the Isopoda and terrestrial Amphipoda, for the axis of the central nervous system is deflected sharply upwards and even bends over a little so that the protocerebrum is dorsal and posterior when compared with the deutocerebrum.

In all, the evidence brought forward in the present chapter indicates that the Crustacea, and probably other Arthropods as well, had originally a ventral mouth, more or less ventral (antennae and) antennulae and a more or less straight central nervous system. Any other supposed arrangement will be inconsistent with the morphological facts. The bearing of this upon the theories put forward by SNODGRASS and others concerning the anterior position of the labrum is obvious but a more detailed discussion will be deferred to the last chapter of the present paper.

It is also obvious that the mode of feeding is one of the influences which have produced the most far-reaching effects upon Crustacean morphology and differentiation.

5. ON THE INTERPRETATION OF THE LABRUM

a. LABRAL MESODERM

The previous chapters dealt almost exclusively with topographical problems, and little was said about the derivation of the various morphological elements concerned. Without belittling the importance of the topography and its adaptational changes the understanding of the process involved demands, however, a somewhat deeper probing into the morphological significance of at least one of the elements dealt with, the labrum.

As demonstrated by various embryologists for various Arthropod groups the labrum is a rather complicated structure. An extensive and critical summary of recent work was given by WEBER (1952) and therefore it is not necessary here to recapitulate more than a few particularly important points.

As shown by TIEGS (1940) for *Hanseniella*, by MANTON (1928) for *Hemimysis* and by other authors for various insects, the labral mesoderm is originally derived from the pre-antennulary mesoderm.

In *Hanseiella*, however, TIEGS found (1940 p. 47) and demonstrated in great and completely convincing detail that during somewhat later ontogenetical stages when the labrum (or rather clypeolabrum) continues to grow downwards along the ventral side, part of the pre-mandibulary mesoderm rolls over into it. Although it had not been actually demonstrated in the case of the Crustacea it was to be expected that a similar process takes place among them, for only in that way could the innervation from the tritocerebrum be satisfactorily explained (cf. p. 181).

In *Artemia salina* I found proof that a process probably similar to that encountered by TIEGS in *Hanseniella* takes place. In late embryos and recently hatched larvae there exists a direct continuation of the mesoderm of the antennar region into the labrum. In serial sections this gives the impression of an immigration of mesodermal as well as nervous material into the basal part of the labrum proper, but it is more probable that what takes place is actually a rolling in of part of the antennar segment caused by the growth of the labrum, as described by TIEGS. But before this communication with the mesoderm of the antennar segment is established there already exists a labral mesoderm. I have not myself investigated its origin, but considering the evidence from *Hemimysis* and other arthropods one may probably safely infer that it is pre-antennular mesoderm, all the more so as no mesodermal material from the antennular segment enters the labrum.

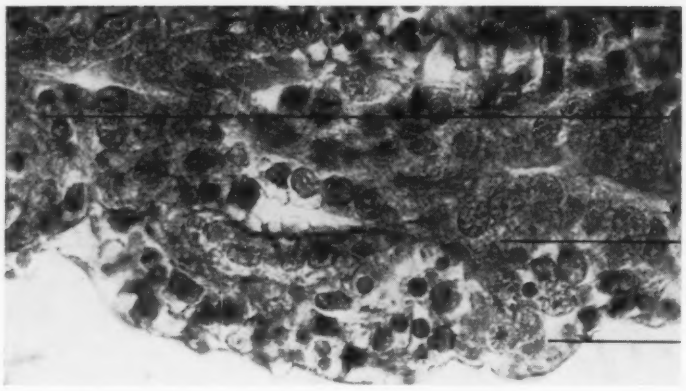

Fig. 31. Recently hatched metanauplius of *Artemia salina* showing how the labrum is at this stage invaded by nervous and mesodermal tissue from the tritocerebral segment. The invasion takes place just where the labrum is joined to the body wall(x). Further explanation in text.

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CHAUDONNERET (1950) produced evidence supporting the view that the labrum in *Thermobia* receives mesodermal tissue from more than one segment, and TIEGS (l.c.) demonstrated this to be the case in *Hanseniella*. WEBER (l.c.) laid great stress upon these results in his attempt to synthesize from the evidence available a coherent picture of the arthropod head, an attempt which produced most valuable results and seems to solve many of the essential and much-debated problems concerned.

There seems, however, to be some difficulties involved in WEBER's views on the mesoderm of the labrum. He writes (l.c. p. 54) "Die auf das Acron folgenden Segmente, das präantennale, das antennale und das prämandibulare (tritocerebrale) bilden bei den Arthropoden und den Onychophoren mit dem Acron zusammen das Procephalon. Sie überflügeln durch Vorwachsen ihrer Seitenteile in der Embryonalentwicklung den Mund, vereinigen sich vor ihm und dem Acron und bilden hier das Clypeolabrum." Setting aside for the present the suggestion that not only the mouth but also the acron is by-passed by the segments growing forward, we find here that all three anterior segments are supposed to take part in the formation of the clypeolabrum. However, it seems doubtful, whether the antennular segment is really concerned in the formation of the labral mesoderm. From TIEGS' description of *Hanseniella* this would not seem to be the case in the Symphyla. In the case of *Hemimysis* MANTON (1928 p. 451) states that "the preantennulary somites grow backwards through the antennulary segment and invest the stomodaeum." In *Artemia* I have seen no evidence of any antennular mesoderm passing into the clypeolabral area (if that expression be permitted in the case of a Crustacean).

Consequently it appears as if the clypeolabrum (or epistome + labrum)

receives its original mesoderm from the preantennular somites and grows backwards past the antennular segment without obtaining any mesodermal elements from it. It may be, though, that the chronology is somewhat different, for in *Hemimysis* the labrum is actually formed behind the antennular somites, but it is not known if there is any transport backwards of ectoderm belonging to it before its actual formation. In *Hanseniella*, on the other hand, the formation of the clypeolabrum starts well in front of the antennae (= antennulae of Crustacea), although its definite position is posterior to them. The larger part of the labral mesoderm is contributed later on by the antennar (tritocerebral, intercalary, premandibular) somites.

Such a by-passing of an entire segment is hardly compatible with the traditional concept of a segment as a section of the body with well-defined limits both in mesoderm and ectoderm layers. But although this concept undoubtedly retains its validity in the larger part of the body, i.e. in the whole area originating from the "post-naupliar" growth zone, grave doubts prevail as to whether it can be applied without considerable modification to the head region. MANTON (1949) made certain important suggestions concerning the relationship between mesoderm and ectoderm in the arthropod head which were elaborated by DAHL (1954) who suggested that in the head region it may be possible and indeed necessary to assume that the ectoderm does not become assigned to corresponding somites until considerably later than in a normal segment, where the two are formed more or less simultaneously, that, in fact there occurs a considerable amount of early movement of mesodermal elements without the ectoderm of the body wall being affected to nearly the same extent as could be expected. This must under no circumstances be understood as a denial of early movements of ectodermal material in the head region for such movement certainly occurs even on a fairly large scale, it merely refers to the mutual relationship between ectoderm and mesoderm. It shall be readily admitted that this hypothesis is still somewhat conjectural, but certain facts were found to support it (cf. DAHL l.c.), and it would seem as if the peculiar formation of the labrum recorded on the previous pages could be much more easily understood by accepting this than the traditional segment concept. Otherwise it will be very difficult to explain how the preantennular somites grow straight through the antennular segment (cf. p. 178).

The participation of the preantennular as well as the antennar somites in the formation of the labral and stomodaeal mesoderm may possibly have a significance affecting the very core of the problem dealt with in the present paper.

As unanimously agreed by all writers on the subject the mouth in the annelid progenitors of the arthropoda must have been subterminal. The exception is HENRY, who, as previously shown, misinterpreted basic morphological facts. This subterminal position implies that the anterior part of the stomodaeum probably got its musculature from the first segment and that the other mesoderm

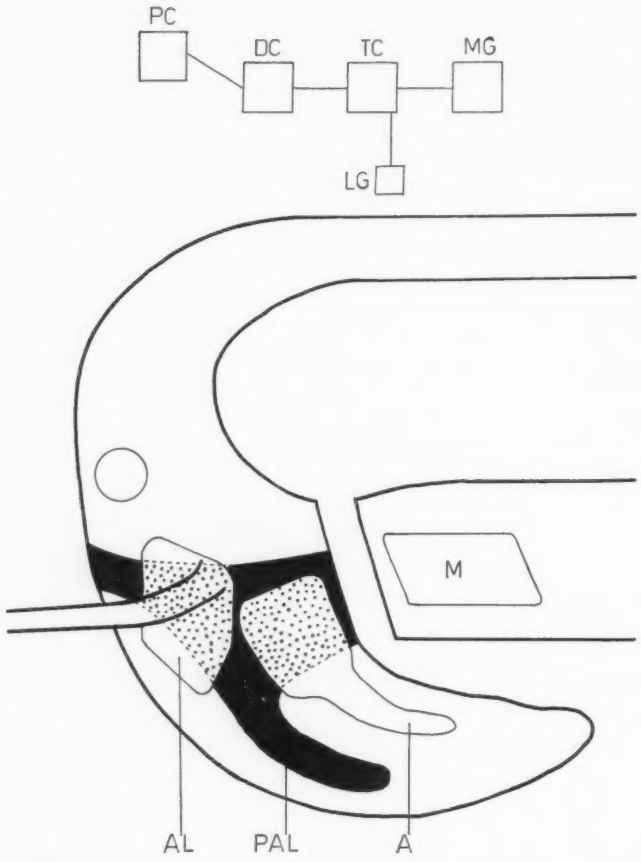

Fig. 32. Diagrammatical view of the arrangement of somites and ganglia (above) in the head of a Crustacean of a primitive filter-feeding type. The somites are not intended to represent the actual shape, only the mutual positions of the respective elements. PAL preantennular, AL antennular, A antennar, M mandibular somites, PC protocerebrum, DC deutocerebrum, TC tritocerebrum, MG mandibular, LG labral ganglion.

of the mouth region was also derived from the first segment. As shown in previous chapters strong evidence supports the view that the mouth opening of early arthropods was dislodged backwards along the ventral side. This dislocation together with the probable prevalence of filter-feeding in any case in the progenitors of the Crustacea and possibly also in other early arthropods brought the labrum into existence and certainly played an important part in the formation of the arthropod head. But it may well be that despite its new position the anterior part of the stomodaeum and the region immediately in front of the mouth retained its connection with the first segment, receiving its mesodermal elements from it. In *Hemimysis* this condition is well illustrated, for as shown

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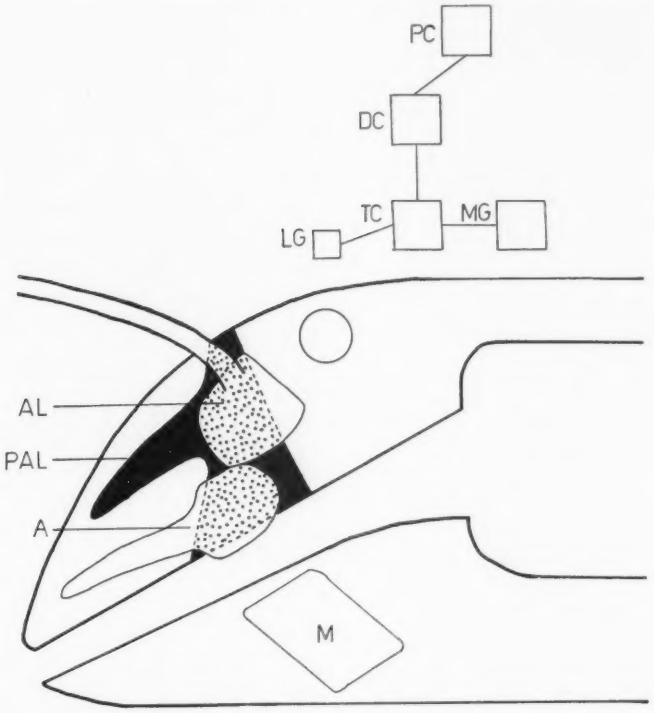

Fig. 33. Diagrammatical view of arrangement of somites and ganglia (above) in the head of a Crustacean of the progmathous type represented by many Isopoda and terrestrial Amphipoda. Lettering as in fig. 33.

by MANTON (1928) much of the musculature of the stomodaeum is formed from the posterior end of the attenuated preantennular somites, the anterior end of which lie far in front of it on the other side of the antennular segment. Another diverticle from each preantennular somite projects into the labrum (MANTON l.c. text fig:s 12—14). But the great development of the labrum also draws in a considerable amount of mesoderm from the somites nearest to its points of attachment, the antennar segment, as described above p. 177. It seems to me that this interpretation accounts reasonably for the available facts.

b. LABRAL INNERVATION

The understanding of the origin of the labral mesoderm is of fairly recent date, but all essential facts concerning its innervation have been known for a long time, and it is enough here to refer to summaries by HANSTRÖM (1928) and WEBER (1952). The labral innervation is tritocerebral, and as far as known at present the pre-antennulary labral mesoderm receives no innervation from the

part of the brain which should correspond to it, the prococephalon in the terminology of WEBER (l.c. p. 55).

The labral nerves have their roots in the posterior part of the tritocerebrum proper or, notably in the case of various Malacostraca, in the so-called connective ganglia on the circumoesophageal connectives behind the tritocerebrum from which they were ontogenetically derived. This arrangement is especially found in larger forms (Mysids, Decapoda) and it may have a very simple explanation. In these large forms the relative volume of the nervous system is much smaller than in small forms, but as they possess a true syn-cerebrum which is located far forwards in the head the circumoesophageal connectives become, both relatively and absolutely, very long. It seems as if the nerve cells corresponding to the labral nerves are simply "held back" in the position they occupied in the larvae and which was then in direct contact with the tritocerebrum proper (cf. figs 28—30 of the larvae of *Leander*.)

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6. CONCLUSIONS

The time has come to regard the facts and considerations presented in the preceding chapters in the light of the various interpretations of the Crustacean head.

I must state from the start that I am not going to discuss in any detail the theories and the alleged results of FERRIS and HENRY. This is not because I refuse to have anything to do with the theories as such, for they have the same right to be considered as any others, but because it seems obvious that they lack the foundation of facts which should make them ripe for discussion. On p. 130 it was pointed out that the interpretation given by HENRY concerning the homologization of the nervous system in Oligochaeta and Polychaeta is entirely arbitrary and most probably erroneous. But worse still, my re-investigation of the nervous system in *Nereis* (which was already very well known although HENRY seems to have been unfamiliar with the most important papers) demonstrated fundamental errors of observation in her "objective description". As HENRY herself points out, the nervous system of *Nereis* constitutes the very basis of her further discussions. "Had almost any other species been examined first, the problem might possibly have been dismissed as insoluble." (HENRY 1947 b. p. 84). FERRIS (1947 p. 63) in his introductory remarks to his pupil HENRY's investigation also lays great stress upon conditions in *Nereis*, and, incidentally, in his somewhat ironical remarks on the ignorance of various writers on the genus amply demonstrates his own unfamiliarity with the existence of the fundamentally important papers by RETZIUS and HOLMGREN. However, as the whole interpretation presented by HENRY collapses with her confusing the tendons and ligaments of the *Nereis* jaws with ganglia and ner-

ves it seems senseless to discuss the further development of her or rather FERRIS' theories. Should new and reliable facts supporting them be presented I shall be prepared for such a discussion. It should be pointed out that WEBER, too, (1952 p. 40) refers to errors of observation on the part of HENRY, though in other parts of her investigations.

I shall not take up here one by one other recent theories on the composition and derivation of the arthropod and especially the crustacean head for they were reviewed by WEBER (l.c.). WEBER's own interpretation (l.c. pp. 51 ff.), which was based in the first place on the important investigations carried out by TIEGS, is the one which most closely agrees with my own views on the matter and here I shall particularly discuss those of my own results which support WEBER's theories or call for modifications of them. Other interpretations will be dealt with more cursorily in the course of the discussion.

It should perhaps be pointed out that I am well aware that what is valid for Crustacea may not hold for Myriapods and Insects or vice versa. As pointed out by various writers there seems to accumulate a vast amount of evidence in favour of the view that the separation of the various arthropod stocks took place very early. It seems, indeed, to be difficult, not to say impossible, to derive a phylogenetic line towards Myriapods and Insects from any known type of Crustacean, fossil or recent, and it seems increasingly probable that STÖRMER (various papers e.g. the monograph of 1944) was right in his assumption that the separation between arachnomorph and mandibulate lines took place already at a pre-trilobite stage of development. Nevertheless the various major groups of mandibulates apparently have got so much of their structural plan in common that it may be justified to let evidence derived from one group support that from others as long as its source is clearly stated.

I think this is important for by elucidating the original structural plan of the Crustacea we should be able to throw some light also on that of Insecta and Myriapoda which may reasonably be supposed to have advanced further from the original primitive arthropod types. Nevertheless most of the literature dealing with the structure of the head in arthropods refers to insects.

It was repeatedly pointed out in the preceding chapters that all available evidence supports the view that the primitive structural plan in the Crustacea shows the following principal features, viz. a mouth opening placed well behind the anterior end of the (functionally) ventral side, with a stomodaeum pointing more or less backwards from the point where it joins the midgut which may be behind the anterior end of the latter, with ventrolateral antennae and ventrolateral or anteroventral antennulae, with protocerebral sense organs more or less terminal and with the axis of the nervous system forming a straight or nearly straight line past the mouth and at least into the deutocerebrum. It was also demonstrated that this pattern is prone to be modified, and it was at least made probable that the main adaptational influence is the mode of life and above all

the mode of feeding and the choice of food. In the Crustacea structurally farthest removed from the primitive pattern described above the mouth is anteroventral, the clypeolabrum facial or dorsal, the antennae and antennulae facial or, in the case of the latter even anterodorsal, the compound eyes dorsolateral and the axis of the central nervous system curving abruptly upwards from somewhere in the tritocerebral region and even tilted over caudally in its terminal part. The Crustacea showing this extreme modification are the Isopoda and the terrestrial Amphipoda of the family *Talitridae*, i.e. the groups harbouring the majority of terrestrial Crustacea and among them those forms which in their choice of habitat, their general mode of life, and their mode of feeding most closely resemble insects or myriapods. It is striking, too, that in their structural plan, as outlined above, they approach more closely that of the average insect or myriapod than do any other Crustacea, with the exception of the aquatic Isopoda. The aquatic Isopoda have—at least in a large number of cases—adopted a mode of feeding closely resembling that of their terrestrial relatives.

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As pointed out on p. 123, SNODGRASS in his later papers (1951, 1952) maintains that the labrum and epistome are "primarily facial or dorsal; the ventral position of these structures in most Crustacea, and in Trilobita and Xiphosurida, is due to their secondary inflection upon the under surface of the head. In the amphipods, isopods, and insects, the epistome (clypeus) is typically facial in position, dorsal or posterior to the labrum. The crustacean epistome, therefore, cannot be the 'sternum' of the antennal or mandibular segments, as it is sometimes said to be by carcinologists." (SNODGRASS 1951 p. 107). With the opinion expressed in the last sentence I can only agree; the previous ones, however, rather appear to turn the available evidence upside-down. They are the outcome of SNODGRASS' opinion that the tip of the labrum is the morphologically anterior end of the mandibulate arthropods. "Embryogeny of the arthropods leaves little doubt that the labrum represents the anterior pole of the animal, and that the epistome is primarily a part of the dorsal or facial surface of the head, as it is in adult amphipods and isopods among the Crustacea, and in the insects." (l.c. p. 3). It is true that the mesoderm originally belonging to the labrum is derived from the pre-antennular somites, which, as far as the available evidence goes, represent the first segment. However, it seems necessary to assume the presence of some sort of acron corresponding in the nervous system to the anterior (and larger) part of the protocerebrum (TIEGS 1940 p. 118, WEBER 1952 pp. 52 ff.). It seems probable that the large prostomium postulated by HOLMGREN and HANSTRÖM must be reduced to the extent suggested by WEBER, but the remaining parts are nevertheless very important, as the corresponding anterior section of the brain innervates the protocerebral sense organs and, in the forms where they occur, include the corpora pedunculata. Even if, with ROONWAL, we should accept the idea that the original archicerebrum has disappeared

altogether and place a labral ganglion in front of the preantennar ganglion (in insects) we still are forced to admit a lobus opticus in front of the middle protocerebral lobe which, according to ROONWAL, should represent the cerebral ganglion of the labral segment.

Thus morphologically the labrum cannot be considered as the anterior pole of the body, for the prostomium or acron lies in front of it, even if it happens to represent the functional anterior end which is often the case. Not even if we accept WEBER's theory (l.c. p. 54) that the preantennar, antennar, and premandibular (in Crustacea the preantennular, antennular, and antennar) segments grow forwards and fuse in front of the mouth and the acron, is there a possibility of the labrum becoming the anterior pole, for, as pointed out by WEBER himself, the part of the acron carrying the protocerebral sense organs becomes separated from the mouth and is pushed upwards. Thus only that part of the acron which lies just in front of the mouth becomes surrounded by originally postoral segments, and the other, separate part, becomes logically the anterior pole (cf. l.c. fig. 8. p. 58). We shall revert later on to these questions, for there I shall suggest an alternative to the opinion held by WEBER.

It is, under these circumstances, rather a dialectic than a morphological question whether the epistome and labrum can be considered to be, under any circumstances, morphologically dorsal. In the Annelida there exists nothing which could be homologized with a labrum. In a form with a terminal mouth the upper margin of the mouth opening is, of course part of the dorsal half of the body. In chapter 3 of this paper reasons were put forward supporting the view that the mouth in the arthropods is not morphologically terminal, that in fact one of the processes operative in the creation of the arthropod types was a dislocation of the mouth towards the ventral side. In the course of that process the labrum was formed.

Other difficulties, too, seem to arise out of the assumptions made by SNODGRASS. Even if he makes a point of not discussing arthropod interrelationships ("Phylogenetic trees are best reared on an eclectic diet" l.c. p. 110) it seems very hard to accept a condition found in highly differentiated forms such as insects (appearing in the Devonian), isopods and amphipods (possibly late Palaeozoic, otherwise Mesozoic) as primary, and the corresponding conditions occurring in Trilobita (early Cambrian or older), Xiphosura (Silurian or older), and primitive Crustacea (probably since early Cambrian) as secondary. To me it seems quite impossible to accept the views on the labrum held by SNODGRASS in his later works.

To revert again, after this digression, to the main issue, it appears, as if both CHAUDONNERET and WEBER experienced some difficulties in explaining the bending upwards of the nervous system in the head of many arthropods, notably insects. The survey made of conditions in the Crustacea in the present paper would seem to offer a fairly simple solution to some of the problems involved by

demonstrating the selective influence of the functional demands which may have been operative in producing such a change. If in the Crustacea we accept the fact that the mouth is still more or less ventral, although retaining its connection by means of the pre-antennular somite with the anterior end of the body, and reckon with a bending upwards, even upwards-backwards of the anterior part of the ventral side in the manner described on pp. 175—176, we obtain in a comparatively simple manner a head of the isopod-amphipod-insect type. There is evidence that the mouth moves backwards on the ventral side both phylogenetically and often also ontogenetically but there is hardly any evidence of a subsequent migration forwards even in prognathous forms, (cf. p. 175) for the antennulae are (from a morphological point of view) situated in front of the mouth (cf. diagrams fig. 33). In the Crustacea we find practically every conceivable intermediate step between the conditions represented in fig. 32 and 33, and we have seen the nervous system being deflected upwards in a corresponding manner.

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It is, of course, an open question whether these considerations may be applied to the phylogenetic line leading to the insects. However, there are some reasons to believe that such an application can be made, viz. 1) The line in question must be originally derived from aquatic forms. 2) As far as known all primitive aquatic arthropods possessed a ventral mouth. 3) The formation of the labral mesoderm from the preantennal and intercalary somites in insects and chilopods is in its essentials identical with the same process in Crustacea. 4) A transition to a terrestrial life and the ensuing changes in the mode of feeding are likely to produce corresponding changes in both groups.

As mentioned above p. 185) WEBER suggests that part of the acron was grown over by the preantennar, antennar, and intercalary segments (preantennular, antennular, and antennar in Crustacean terminology). Without wishing to offer any criticism of this theory, I would suggest a modification which may be an alternative. If we accept the possibility that the ectoderm of the head region is not from the beginning so definitely tied to mesodermal somites as in the posterior part of the body (cf. DAHL 1954, also above p. 179), it might be possible that the three pairs of somites grow forwards past the mouth without any corresponding ectodermal parts being drawn forwards to envelop the ventral part of the acron.

It is also a possibility, which deserves to be considered, that the topographical changes taking place in a developing arthropod embryo consist not so much in a moving forwards of post-oral segments as a recapitulation of the movement of the mouth in a posterior direction. This opens the possibility for the mouth located in the first (preantennular) segment to move backwards, retaining its position in the same segment and figuratively speaking pushing aside the elements belonging to the antennulary and antennary segments without being enveloped by them. As shown by TIEGS' drawings something of this sort

actually happens in *Hanseniella*. The matter requires thorough investigation in Crustacea. The drawing in of mesodermal material from the antennar (intercalary) segment into the labrum is explained in the same way as before by its great development, and this, incidentally, will also conform with the idea of a recapitulation.

It seems to me that either of these two possibilites support the views put forward by WEBER at the same time as they remove the need for the part of them which seems hardest to accept, viz. the enveloping of part of the acron by postoral ectoderm. They also offer a perfectly good possibility for a reconciliation with the theories of HOLMGREN and HANSTRÖM, in a modified form accepting the postoral origin of the preantennular, antennular, and antennar somites.

Throughout this paper stress has been laid upon the adaptations necessary to produce out of an annelid progenitor a primitive, filterfeeding arthropod, and out of the latter a rapacious, scavenging or herbivorous type. Attention has been called to the close interaction between the mode of feeding and adaptational morphological change in those parts of the long chain, notably among the Crustacea, which are available for investigation. It seems a reasonable assumption that the same forces were in action in those parts, which, owing to the scantity of the fossil record, are withdrawn from our observation. In short, it seems as if a close study of the interaction between mode of life and morphological transformations offers solutions and sometimes fairly simple solutions to many of the problems otherwise inaccessible.

7. EXPLANATION OF ABBREVIATIONS USED IN FIGS 2—31

ao	atrium oris
br	brain
c	connective
ce	compound eye
dc	deutocerebrum
dcg	deutocerebral glomeruli
j	jaw
l	labrum
lig	ligament
m	mandible
mg	midgut
na	antennar nerve
nal	antennular nerve
ne	nauplius eye
no	optic nerve
pc	protocerebrum
r	rostrum
st	stomodaeum

tc	tritocerebrum
v	ventricle
vch	ventral nerve chain
x	explanation in legend of respective fig.

8. REFERENCES

AGAR, W. E., 1908. Note on the early development of a Cladoceran (Holopedium gibberum). Zool. Anz. vol. 33. Lepizig.

BACKLUND, H. O., 1945. Wrack fauna of Sweden and Finland. Ecology and Chorology. Opusc. Ent. Suppl. 5. Lund.

BALSS, H., 1938. Stomatopoda in Bronn: Klassen u. Ordn. vol 2 Abt. 1 b. 6:2. Leipzig.

— 1940. Decapoda, Lief. 1. Ibid. b. 7.

— 1944. D:o Lief. 3—4. Ibid.

BLEGVAD, H., Om nogle Danske Gammariders og Mysiders Biologi. Diss. inaug. København.

BORG, F., 1935. Zur Kenntniss der Cladoceren-Gattung Anchistropus. Zool. Bidr. fr. Uppsala. bd. 15. Uppsala.

BORRADAILE, L. A., 1917. On the structure and function of the mouthparts of the Palaemonid Prawns. Proc. Zool. Soc. 1917. London.

— 1922. On the mouth-parts of the Shore Crab. J. Linn. Soc. vol. 25. London.

BREHM, V., 1927. Copepoda, in Kükenthal-Krumbach: Handb. d. Zool. vol 3:1. Berlin and Leipzig.

CANNON, H. G., 1922. On the Labral Glands of a Cladoceran (Simocephalus vetulus) with a description of its mode of feeding. Quart. J. Micr. Sci. vol. 66. London.

— 1926. On the feeding of a fresh-water Ostracod, Pionocypris vidua (O. F. Müller). J. Linn. Soc. vol. 36. London.

— 1927. On the feeding mechanism of Nebalia bipes. Trans. Roy. Soc. Edinburgh. vol. 55.

— 1928 a. The feeding mechanism of the fairy shrimp, Chirocephalus diaphanus. Ibid.

— 1928 b. On the feeding mechanism of the Copepods, Calanus finmarchicus, and Diaptomus gracilis. Brit. J. Exp. Biol. vol. 6. London.

— 1931. On the anatomy of a marine Ostracod, Cypridina (Doloria) levis Skogsberg. Discovery Rep. vol. 2. Cambridge.

— 1940. On the anatomy of Gigantocypris mülleri. Ibid. vol. 19.

— 1947. On the anatomy of the pedunculate barnacle Lithotrya. Phil. Trans. Roy. Soc. B. vol. 233. London.

— and MANTON, S. M., 1927. On the feeding mechanism of a Mysid Crustacean. Hemimysis Lamornae. Trans. Roy. Soc. Edinb. vol. 5.

— and MANTON, S. M., 1929. On the feeding mechanism of Syncarid Crustacea. Ibid. vol. 56.

CHAPPUIS, P. A., 1927. Anaspidacea, in Kükenthal-Krumbach Handb. der Zool vol. 3:1, Berlin and Leipzig.

— and DELAMARE DEBOUTTEVILLE, C., 1953. Morphologie des Mystacocarides. Arch. Zool. Exp. Gen. t. 91:1. Paris.

CHAUDONNERET, M. J., 1946. La morphologie céphalique de Thermobia domestica (Packard), insecte apterygote thysanoure. Ann. Sci. Nat. Zool. ser. 11 t. 12. Paris.

CHUN, C., 1896. Atlantis, biologische Studien über pelagische Organismen. Bibliotheca Zoologica bd. 7. Stuttgart.

VOL. 37 1956

CLAUS, C. 1873. Zur Kenntniss der Baues und der Entwicklung von Branchipus stagnalis und Apus cancriformis. Abh. K. Ges. Wiss. Göttingen bd. 18.
— 1889. Ueber den Organismus der Nebaliden und die systematiche Stellung der Leptostraken. Arb. Zool. Inst. Wien. tom. 7.
DAHL, E. 1952. Mystacocarida. Rep. Lund Univ. Chile Exp. 7. Acta Univ. Lund (= Lunds Univ. Årsskr.) N. F. Avd. 2, Bd 48:6 Lund.
— 1953. Frontal organs in free-living Copepods. K. Fysiogr. Sällsk. Förh. bd 23:5. Lund.
— 1954. To the question of larval segments and brain structure and their bearing upon Crustacean phylogeny. Ibid. bd 24:6.
DELAMARE DEBOUTTEVILLE, C., cf. Chappuis.
DELLA VALLE, A., 1893. Gammarini del Golfo di Napoli. F. Fl. d. Golfes v. Neapel. monogr. 20. Berlin.
DELSMAN, H. C., 1917. Die Embryonalentwicklung von Balanus balanoides. Tijdschr. Nederl. Dierk. Ver. ser. 2 d. 15. Leiden.
DENNELL, R., 1934. The feeding mechanism of the Cumacean Crustacean Diastylis bradyi. Trans. Roy. Soc. Edinb. vol. 58.
DEPDOLLA, Ph., 1923. Nahrung und Nahrungserwerb bei Praunus flexuosus (Müll.) Biol. Centrlbl. bd 43. Leipzig.
ENEQUIST, P., 1949. Studies on the soft-bottom Amphipods of the Skagerak. Zool. Bidr. fr. Uppsala, bd. 28. Uppsala.
ERIKSSON, S., 1928. Ueber den Fangapparat von *Sida crystallina*. Ibid. bd. 12.
— 1934. Studien über die Fangapparate der Branchipoden nebst einigen phylogenetischen Bemerkungen. Ibid. bd. 15.
FERRIS, G. F., 1947. The contradictions of the insect head. Microentomol. vol. 12:3. Stanford.
FORSMAN, B., 1938. Untersuchungen über die Cumaceen des Skageraks. Zool. Bidr. fr. Uppsala vol. 19. Uppsala.
FOXON, G. E. H., 1936. Notes on the natural history of certain sanddwelling Cumacea. Ann. Mag. Nat. Hist. ser. 10 vol. 17. London.
FRÄNSENMEIER, L., 1939. Zur Frage der Herkunft des metanauplialen Mesoderms und die Segmentbildung bei Artemia salina Leach. Zeitschr. Wiss. Zool. bd. 152. Leipzig.
FUCHS, K., 1914. Die Keimblätterbildung von Cyclops viridis Jurine. Zool. Jahrb. Anat. bd. 38. Jena.
HAMAKER, J. I., 1898. The nervous system of Nereis virens Sars. Bull. Mus. Comp. Zool. Harvard. vol. 32.
HANSTRÖM, B., 1926. Das Nervensystem und die Sinnesorgane von Limulus polyphemus. Acta Univ. Lund. (= Lunds Univ. Årsskr.) N. F. Avd. 2 bd 22:5. Lund.
— 1928. Vergleichende Anatomie des Nervensystems der Wirbellosen Tiere. Berlin, Julius Springer.
— 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. Zeitschr. f. Morph. bd. 23 Berlin.
— 1933. Neue Untersuchungen ... II. Zool. Jahrb. Anat. bd. 56. Jena.
— 1934. Neue Untersuchungen ... IV. Arkiv f. Zool. bd 26 A. Stockholm.
HENRY, L. M., 1947 a. The nervous system and the segmentation of the head in the Annulata. Introduction and II. Oligochaeta. Microentomology vol. 12:3. Stanford.
— 1947 b. D:o. III. Polychaeta. Ibid. vol. 12:4.
— 1948 a. D:o IV. Arthropoda. Ibid. vol. 13:1.
— 1948 b. D:o. V-VII. Onychophora. Chilopoda, Insecta. Ibid. vol. 13:2.
HICKMAN, V. V., 1937. The embryology of the Syncarid Crustacean, Anaspides tasmaniae. Pap. Proc. Roy. Soc. Tasm. 1936. Hobart.

HOLMGREN, N., 1916. Zur vergleichenden Anatomie des Gehirns von Polychaeten, Onychophoren, etc. K. Sv. Vet. Ak. Handl. bd. 56: 1. Stockholm.

HULT, J., 1941. On the soft-bottom Isopods of the Skagerak. Zool. Bidr. fr. Uppsala. bd. 21. Uppsala.

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. J. Mar. Biol. Ass. vol. 23. Plymouth.

IVANOV, P. P., 1928. Die Entwicklung der Larvalsegmente bei Anneliden. Zeitschr. f. Morph. vol. 10. Berlin.

JACOBS, M., 1925. Entwicklungsphysiologische Untersuchungen am Copepodenei (Cyclops viridis Jurine). Zeichschr. Wiss. Zool. bd. 124. Leipzig.

JONES, L. L., 1936. A study of the habitat and habits of Emerita emerita. Proc. Louisiana Ac. Sci. vol. 3. Baton Rouge.

KORSCHELT and HEIDER, 1936. Vergleichende Entwicklungsgeschichte der Tiere. Revised by E. Korschelt. Jena, Gustav Fischer.

KÜHN, A., 1913. Die Sonderung der Keimbezirke in der Entwicklung der Sommereier von Polyphemus pediculus de Geer. Zool. Jahrb. Anat. bd. 35. Jena.

LANG, K. 1948. Monographie der Harpacticiden. Stockholm, Nordiska Bokhandeln.

LEBOUR, M. V. 1924. The Euphausiidae in the Neighbourhood of Plymouth and their importance as herring food. J. Mar. Biol. Ass. vol. 13. Plymouth.

LUNDBLAD, O., 1916. Om ett fynd av Limnadia lenticularis (L) i Sverige. Zool. Bidr. fr. Uppsala bd. 4. Uppsala.

— 1920. Vergleichende Studien über die Nahrungsaufnahme einiger schwedischen Phyllopoden. Arkiv f. Zool. bd. 13. Stockholm.

MACGINITIE, G. E. and N., 1949. Natural history of marine animals. New York, MacGraw-Hill.

MANTON, S. M. 1928. On the embryology of a Mysid Crustacean, Hemimysis Lamaornae Phil. Trans. Roy. Soc. B. vol. 216. London.

— 1930. Notes on the habits and feeding mechanisms of Anaspides and Paranaspides (Crustacea, Syncarida), Proc. Zool. Soc. London, 1930 pt 3—4.

— 1934. On the embryology of the Crustacean Nebalia bipes. Phil. Trans. Roy. Soc. B. vol. 223. London.

— 1949. Studies on the Onychophora. VII. The early embryonic stages of Peripatopsis etc. Ibid. vol. 233.

MANTON, S. M. and CANNON, H. G. vide Cannon.

MEAD, A. D., 1897. The early development of Marine Annelids. J. Morph. vol. 13. Boston.

MÜLLER-CALÉ, C. 1913. Ueber die Entwicklung von Cypris incongruens. Zool. Jahrb. Anat. bd. 36. Jena.

NICHOLLS, G. E. 1931. Micraspides calmani, a new Syncaridan from the west coast of Tasmania. J. Linn. Soc. vol. 37. London.

— and SPARGO, D., 1932. Notes on the internal anatomy of the Anaspidacea. Ann. Mag. Nat. Hist. ser 10 vol. 10. London.

PFISTERER, O. and STORCH, O. vide Storch, O.

RAW, F., 1949. Some stages in the evolution of the nervous system and the fore-gut of the polychaet. Smiths. Misc. Coll. vol. 111: 8. Washington.

REICHENBACH, H., 1886. Studien zur Entwicklungsgeschichte des Flusskrebses. Abh. Senck. Ges. bd. 14.

RETZIUS, G., 1895. Zur Kenntnis des Gehirnganglions und des sensiblen Nervensystem der Polychaeten. Biol. Unters. N. F. bd. 7. Stockholm.

RICHARD, J., 1891. Recherches sur le système glandulaire et sur le système nerveux des Copépodes libres d'eau douce. Ann. Sci. Nat. ser. 7, Zool. t. 12. Paris.

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ROONWAL, M. L., 1936. Studies on the embryology of the African migratory locust, Locusta migratoria migratoricides, R. and F. (Orthoptera, Acrididae). I. Phil. Trans. Roy. Soc. B. vol. 226. London.

— 1937. D:o, II. Ibid. vol. 227.

— 1938. Some recent advances in insect embryology, with a complete bibliography of the subject. J. Roy. Asiat. Soc., Sci. vol. 4.

SCOURFIELD, D. J., 1926. On a new type of Crustacean from the Old Red Sandstone (Rhynie Chert Bed, Aberdeenshire) — Lepidocaris rhyniensis, gen. et sp. nov. Phil. Trans. Roy. Soc. B. vol. 214. London.

SIEWING, R., 1951. Besteht eine engere Verwandschaft zwischen Isopoden und Amphipoden? Zool. Anz. bd. 147. 1951.

SMITH, G., 1908. Preliminary account of the habits and structure of Anaspididae etc. Proc. Roy. Soc. London. ser. B. vol. 80. London.

— 1909. The Anaspidacea, living and fossil. Quart. J. Micr. Sci. vol. 53. London.

SNODGRASS, R. E., 1938. Evolution of the Annelida, Onychophora, and Arthropoda, Smiths. Misc. Coll. vol. 87. Washington.

— 1950. Comparative studies on the jaws of mandibulate arthropods. Ibid. vol. 116.

— 1951. Comparative studies on the head of mandibulate arthropods. Ithaca, Comstock Publ. Co.

— 1952. A textbook of arthropod anatomy. Ithaca, Comstock Publ. Ass.

SPARGO, D. and NICHOLLS, G. E. vide Nicholls.

STORCH, O., 1922. Der Fangapparat der Daphnien für Nannoplankton. Verh. Deutsch. Zool. Ges., bd. 27.

— 1924. Morphologie und Physiologie des Fangapparates der Daphniden. Ergebn. u. Fortschr. d. Zool. Bd. 6.

— 1925 a. Der Phyllopoden-Fangapparat. I. Intern. Rev. d. ges. Hydrobiol. vol. 12.

— 1925 b. D:o II. Ibid. vol. 13.

— 1929. Analyse der Fangapparate niederer Krebse auf Grund von Mikro-Zeitlupenaufnahmen. Biologia Generalis. bd. 5.

STORCH, O. and PFISTERER, O., 1925. Der Fangapparat von Diaptomus. Zeitschr. f. vergl. Physiol. bd. 3.

STØRMER, L., 1944. On the relationships and Phylogeny of fossil and recent Arachnomorpha. Skr. Norske Vidensk.-Ak, Oslo. I Mat.-Naturv. kl. 1944 no. 5.

TAUBE, E., 1915. Beiträge zur Entwicklungsgeschichte der Euphausiden. II. Zeitschr. f. Wiss. Zool. bd. 114. Leipzig.

TIEGS, O. W., 1940. The embryology and affinities of the Symphyla, based on a study of Hanseniella agilis. Quart. J. Micr. Sci. vol. 68. London.

— 1945. The post-embryonic development of Hanseniella agilis (Symphyla). Ibid. vol. 85.

— 1947. The development and affinities of the Pauropoda, based on a study of Pauropus silvaticus. Ibid. vol. 88.

URBANOWICZ, F., 1884. Zur Entwicklungsgeschichte der Cyclopiden. Zool. Anz. bd. 7. Leipzig.

VANDEL, A., 1949. Généralités, composition de l'embranchement, in Grassé: Traité de Zool. t. 6. Paris.

WEBER, H. 1952. Morphologie, Histologie und Entwicklungsgeschichte der Arthropoden. Fortschr. d. Zool. N. F. b. 9. Tübingen.

WESENBERG-LUND, C., 1937. Ferskvandsfaunaen biologisk belyst. II. København, Gyldendalske Boghandel.

WOLTERECK, R., 1913. Ueber Funktion, Herkunft und Entstehungsursachen der sog. "Schwebe-Fortsätze" pelagischer Cladoceren. Zoologica, bd. 26. Stuttgart.

ZIMMER, C., 1913. Untersuchungen über den inneren Bau von Euphausia superba Dana. Ibid.
— 1927 a. Tanaidacea, in Kükenthal-Krumbach Handb. d. Zool. bd. 3: 1 Berlin and Leipzig.
— 1927 b. Isopoda. Ibid.
— 1933. Beobachtungen an lebenden Mysidaceen und Cumaceen. Sitzber. Ges. Naturf. Freunde. Berlin. Jg. 1932.

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L'OVOGENESE CHEZ PHASCOLOSOMA VULGARE
I. DEFINITION CYTOLOGIQUE DES STADES DE CROISSANCE DES OVOCYTES

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I. INTRODUCTION

On trouve dans le liquide coelomique des Sipunculides des cellules reproductrices libres à différents stades d'évolution. M. le Professeur TEISSIER, directeur de la Station Biologique, avait attiré notre attention sur ce fait. Ce matériel nous a semblé présenter des caractères favorables pour une étude physiologique de la croissance des ovocytes. Mais, avant d'entreprendre ces recherches, il était indispensable, par des méthodes cytologiques classiques, de préciser le stade de libération des ovocytes et de définir éventuellement les étapes caractéristiques de leur croissance ultérieure. Ce travail, effectué sur une espèce abondante à Roscoff, Phascolosoma vulgare (Blainv.), nous a en effet permis de déterminer des stades de croissance très nets, au nombre de 6, que nous avons appelés dans l'ordre: o, 1, T, 2, 3 et M*. Le compte rendu de nos recherches cytologiques, objet de cette publication, sera donc essentiellement orienté vers la définition précise de chacun de ces stades et la possibilité de leur reconnaissance sur le vivant. Ils furent, en effet, les points de repère fondamentaux de nos études ultérieures sur le métabolisme respiratoire.

Les cellules reproductrices sont, dans le coelome, mêlées à de nombreux éléments figurés; hématies, leucocytes de plusieurs lignées, urnes, vésicules. Ces éléments ont été souvent étudiés (VOLKONSKY & MARCOU 1933, THOMAS 1932), mais les gamètes n'ont été l'objet que d'observations fragmentaires; à

* 1, 2 et 3 sont les stades de croissance les plus importants. o désigne les ovocytes ovariens, T est un stade transitoire, et M désigne les ovocytes mûrs.

notre connaissance aucune gamétogenèse n'est décrite de façon approfondie chez les Sipunculides. En ce qui concerne le genre Phascolosoma nous signalerons cependant les travaux d'embryologie de GEROULD et les recherches de ANDREWS, BRACHET (1937), CUENOT, HERUBEL, publications dans lesquelles on trouve quelques indications concernant l'ovaire ou les ovocytes.

La libération des cellules reproductrices femelles dans le coelome s'effectue, à peu de chose près, au moment où elles passent du stade d'ovogonie à celui d'ovocyte. Nous comprenons sous le terme d'ovogonie les cellules reproductrices femelles en cours de multiplication mitotique puis évoluant sans accroissement cytoplasmique notable vers le stade diplotène de la prophase meïotique. Le terme d'ovocyte de premier ordre, ou plus brièvement d'ovocyte, sera réservé aux cellules parvenues en prophase de meïose et effectuant leur grand accroissement. C'est donc au cours de cette dernière phase que, chez le Phascolosome, les cellules sont libres dans le coelome. Sa croissance terminée l'ovocyte entre en métaphase de meïose ; il est devenu un oeuf mûr et la ponte a lieu par l'intermédiaire des néphridies.

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II. TECHNIQUES ET IDENTIFICATION DES ELEMENTS INTRACELLULAIRES

1. TECHNIQUES

Récolte. Les animaux sont récoltés à marée basse à Roscoff ou à Terenez dans le sable vaseux des herbiers à Zostera marina.

Elevage. Nous avons pu conserver des Phascolosomes en élevage longtemps, au moins un an, en excellent état. Il suffit de disposer d'une installation permettant à l'eau de mer de ruisseler seulement à la surface du sable (fig. 1). En effet si le sable est recouvert d'une couche appréciable d'eau de mer, les animaux viennent en surface, leur corps se boursoufle par places et l'infection se développe rapidement.

Détermination du sexe. Aucun caractère externe ne permet de distinguer les 2 sexes. Il faut donc prélever une certaine quantité de liquide coelomique et rechercher des cellules reproductrices sous le microscope. On notera toutefois que pendant la pleine saison de reproduction certains mâles ont un aspect laiteux qui permet de les reconnaitre aisément.

Observations sur le vivant. Les animaux sont ouverts en sectionnant la partie postérieure. Le liquide étant recueilli dans une salière (½ à 1 ml. pour un animal de taille moyenne), on détermine, s'il s'agit d'une femelle, à quel stade sont les ovocytes. Le liquide coelomique est ensuite dilué par l'eau de mer, les cellules sont lavées et triées par décantation, on les examine dans une lame creuse ou entre lame et lamelle maintenues écartées par des petits tampons de plastiline.

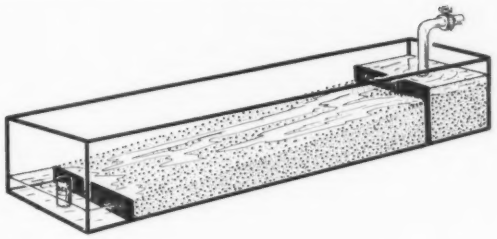

Fig. 1. Installation pour l'élevage prolongé de Phascolosoma vulgare en eau de mer courante.

Nous avons effectué de nombreuses observations sans coloration ou après coloration au rouge neutre ou au vert janus. Le vert janus, en solution au 1/10.000 ème dans l'eau de mer colore certains granules dans les ovogonies et les ovocytes ovariens ainsi que dans les ovocytes libres de stade 1 et 3 (fig. 5 et 6). Mais dans les ovocytes de stade T et 2 la coloration ne se développe pas ou reste diffuse dans toute la cellule. Après des expériences de centrifugation dans un mélange de saccharose et d'eau de mer (GONSE 1955), nous avions observé une excellente coloration par le vert janus de ces stades difficiles. Une étude ultérieure nous a montré que les solutions isotoniques de saccharose provoquent un gonflement de la membrane vitelline ce qui facilite probablement la pénétration du colorant. Nous avons donc traité les cellules par un mélange de saccharose isotonique et d'eau de mer pendant 30 minutes avant de les transférer (braking pipette) dans une solution au 1/10.000 ème de vert janus dans l'eau de mer. La coloration se développe alors en 45 minutes environ, bien limitée à certains granules et sans aspect diffus (fig. 11).

Fixations et colorations. L'ovaire est un organe assez fragile. Nous préférons donc injecter le fixateur dans le coelome, l'animal est disséqué une fois mort et la fixation de l'organe est complétée après son prélèvement. L'étude anatomique a été faite après fixation d'une heure au Helly, sur des coupes à 8 μ d'épaisseur colorées à l'Azan Heideinhain ou au trichromique de Masson.

Pour l'étude des ovocytes nous avons utilisé les méthodes suivantes :

— Fixation au Helly, au Regaud 1 heure, coupes de 6 à 12 μ ; coloration à l'hématoxyline de Regaud, au Feulgen, au vert de méthyle pyronine (tampon acétate-véronal, pH 4,7), au MacManus (periodic acid Schiff), à la gomme iodée. Pour le vert de méthyle pyronine on a effectué le test à la ribonucléase cristallisée, l'extraction par CCl_3COOH, HCl. Pour le MacManus les tests à la salive, à la pectinestérase (Pectolase G. V., Grindstedværket, Danemark), l'extraction par la pyridine.

— Fixation au Champy ou au Benoit (12 à 24 heures à 0°) ; coupes à 3 ou 6 μ ; coloration à la fuschine anilinée d'Altmann.

— Fixation au formol-calcium ; inclusion à la gélose ; coupes à congélation et coloration au noir soudan ou au bleu de Nil.

— Fixateur de Serra ; coloration de Millon-Serra, diazotation. Pour l'appli-

cation de ces techniques nous avons suivi les indications données par: GLICK, LANGERON, LISON, PANIJEL, SERRA.

2. IDENTIFICATION DES ELEMENTS INTRACELLULAIRES

Une étude comparée des résultats obtenus par ces diverses techniques permet de définir dans les ovocytes un certain nombre d'éléments et de suivre leur évolution au cours de la croissance. Avant d'étudier celle-ci, stade par stade, précisons les caractéristiques cytologiques de ces éléments.

Les chromosomes ont été étudiés dans l'ovaire, sur des coupes transversales où les différents stades d'evolution apparaissent bien sériés (fixation au Helly ou au sublimé acétique; coupes à 8—10 μ; coloration à l'hématoxyline de Regaud — vert lumière ou au Feulgen). La morphologie des chromosomes peut être précisée après fixation au sublimé acétique par injection dans le coelome, dissection dans l'orcéine acétique et écrasement (squash) de petits fragments d'ovaire entre lame et lamelle; des mitoses goniales permettent une numération ($2N = 20$).

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Les nucléoles sont bien colorés par l'hématoxyline de Regaud. La pyronine permet d'apprécier les variations de forme et de colorabilité (fig. 12 c). Ces 2 techniques donnent des résultats comparables à ce que l'on observe sur le vivant.

Mitochondries. Sur le vivant, dans les conditions déjà définies, le vert janus colore très nettement des petits granules d'un demi micron environ rassemblés en amas plus ou moins importants (fig. 5 et 6). Seule cette méthode permet de suivre, sans interruption, le chondriome à toutes les étapes de la croissance. Etant donné la spécificité de cette technique (COOPERSTEIN et coll.) nous l'avons considérée comme un témoin pour l'appréciation des résultats obtenus sur coupes.

Ceux-ci, en effet, sont fragmentaires (fig. 17). L'Altmann, après fixation au Champy ou au Benoit, ne colore des amas de type mitochondrial qu'au cours des stades 1 et T (fig. 8 b, 10 c); aucun résultat n'est obtenu aux stades ovariens, tandis qu'aux stades ultérieurs, 2 et 3, la coloration est limitée à des petits granules isolés, peu nombreux, sans rapport avec les amas colorés par le vert janus. Mais, à ces stades 2 et 3, la pyroninophilie du cytoplasme a beaucoup diminué et la coloration par la pyronine se limite à des amas de granules d'aspect tout à fait mitochondrial (fig. 12 a). Le noir soudan donne d'assez bons résultats à tous les stades; l'hématoxyline de Regaud aux stades 1 et T seulement.

La définition du chondriome sur coupes présente donc quelques difficultés,

d'autant plus qu'une technique comme le MacManus, particulière aux polysaccharides mais dont la spécificité est assez faible (Lillie), fournit à tous les stades, en ce qui concerne la localisation des granules, des résultats très comparables à ceux que donne une technique mitochondriale classique comme l'Altmann. Cependant nous avons montré qu'il y a bien 2 groupes de granules de densité différente qui, séparés par centrifugation, fournissent, l'un la réaction des polysaccharides (MacManus), l'autre, celle des mitochondries (vert janus, Altmann) (Gonse 1955).

En résumé, nous avons donc admis que les mitochondries sont les amas de granules colorés à tous les stades par le vert janus, aux stades 1 et T par l'Altmann, aux stades 2 et 3 par la pyronine.

Polysaccharides. L'application de la technique à l'acide periodique Schiff permet de reconnaître dans les ovocytes un certain nombre d'éléments dans la constitution desquels entreraient des polysaccharides.

La membrane vitelline et certains granules cytoplasmiques sont très vivement colorés par cette méthode (fig. 8 d). La réaction n'est pas modifiée par le traitement à la salive (1 heure à 37°). Granules et membrane vitelline ne sont pas colorés par la gomme iodée.

Il semble vraisemblable qu'au niveau de la membrane vitelline la coloration soit due à la présence de muco-protides. En ce qui concerne les granules cytoplasmiques on pourrait rapprocher ces résultats de ceux obtenus sur les oeufs de certains mollusques et penser qu'il s'agit de galactogène. Nous avons donc essayé de tratiter les coupes par une polyestérase-polygalacturonase (Pectolase) dans les conditions indiquées par Grainger & Shillitoe, mais sans résultat net, la colorabilité des granules n'ayant pas sensiblement diminué après ce traitement.

Nous pensons cependant que le galactose doit être l'élément principal de ce polysaccharide granulaire et qu'il est présent également dans la membrane vitelline, car nous avons trouvé, au cours de recherches chromatographiques, des quantités prépondérantes de ce sucre dans les cellules coelomiques de femelles. L'étude analytique du plasma qui sera l'objet d'une publication ultérieure nous a conduit à recueillir et chromatographier des culots de centrifugation. Nous avons ainsi comparé l'ensemble des cellules coelomiques d'animaux femelles (ovocytes, hématies, etc.) au mélange des éléments coelomiques de mâles (spermatozoïdes, hématies, etc), des différences éventuelles entre les 2 séries de chromatogrammes nous permettant d'apprécier ce qui proviendrait des ovocytes proprement dits. On voit que le produit d'hydrolyse des cellules coelomiques femelles est caractérisé par une teneur élevée en galactose et par la présence de ribose qui sont l'un et l'autre absents des cellules coelomiques de mâles. Les indications quantitatives se rapportent à la surface des taches et sont arbitraires.

Composés*	Mâles	Femelles
galactose	—	++++
glucose	++	++
ribose	—	+
désoxyribose	++	++
nucléotide (M1)	—	—
nucléotide (M2)	+	+
nucléotide (N)	++++	+

Rappelons enfin que les granules cytoplasmiques colorables au MacManus se stratifient aisément dans la cellule, se séparant ainsi nettement des mitochondries. Cette densité élevée les apparente bien à un polysaccharide.

Outre la réaction très nette du P.A.S. au niveau des granules et de la membrane vitelline on observe une coloration rose pâle du fond. Au stade 3 apparaît, de plus, une zone périnucléaire fortement colorée (fig. 14 b). Cette dernière coloration, comme celle du fond, ne s'observe plus après action de la salive. La technique à la gomme iodée montre, en brun foncé sur fond jaune pâle, cette zone périnucléaire et colore également très nettement de rares amas mitochondriaux (1 ou 2 par coupe) et quelques granules corticaux. Cette coloration brune est également abolie par le traitement à la salive. Il faut donc admettre l'apparition au stade 3 d'un nouveau polysaccharide qui se présente non plus sous forme granulaire mais diffuse et dont les caractéristiques histochimiques se rapprochent plutôt de celles du glycogène.

En résumé on reconnaîtra donc l'existence de 3 sortes d'éléments : un polysaccharide granulaire (probablement du galactogène) ; un muco-protide présent dans la membrane vitelline ; et, au cours du stade 3 seulement, un polysaccharide sous forme diffuse (probablement du glycogène).

Lipides. Les techniques cytologiques que nous avons utilisées ont permis de distinguer plusieurs sortes d'inclusions lipidiques.

Une première catégorie apparaît en vert foncé ou noir après coloration au noir soudan (fixation au Helly ou au formol-calcium). Ce sont des amas de granules dont l'aspect et l'évolution font penser qu'il s'agit des mitochondries.

D'autres inclusions sont bien mises en évidence par le noir soudan ou par l'hématoxyline de Regaud. Ce sont des lipides de réserve sous forme d'inclusions sphériques d'une taille supérieure, dès leur apparition (1 μ), à celle des mito-

* Cette étude a été faite au mois de Juillet. Les chromatogrammes ont été effectués après hydrolyse acide (SO_4H_2, 0,3 N, 8 heures), dans 2 systèmes de solvants : butanol-ac.acétique-eau 4-1-5 et s-collidine saturée d'eau. M_1, M_2, et N sont des produits d'hydrolyse incomplète d'acides nucléiques.

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chondries ; leur diamètre s'accroît ensuite pour atteindre 4 à 5 μ dans les gros ovocytes.

Enfin dans les cellules de stade 2 et 3 on remarque des petits granules, des bâtonnets, ou des filaments, colorés en noir intense par le noir soudan. Le rouge neutre, sur le vivant, donne des aspects tout à fait semblables. Mais il est difficile de préciser s'il s'agit d'un vacuome, ou peut-être, en partie d'éléments mitochondriaux.

Plaquettes vitellines. Leur observation est difficile. Elles n'apparaissent que tardivement, peu avant la ponte des ovocytes ; elles sont peu nombreuses (quelques dizaines par cellule) et dispersées parmi les inclusions lipidiques. A l'exception du cas très particulier d'ovocytes probablement sur-développés dont nous reparlerons plus loin (stade 3), nous n'avons pas pu reconnaître les plaquettes vitellines sur coupes, mais seulement in toto, au pôle centripète de l'ovocyte, après stratification intracellulaire et fixation par l'acide osmique. Ces plaquettes sont allongées et aplaties, en forme d'ellipse (8 μ sur 4 μ environ). Les colorants des protéines ne nous ont pas donné de résultats nets sur des ovocytes susceptibles de contenir des plaquettes.

Pyroninophilie. La technique au vert de méthyle pyronine nous a permis de constater l'affinité variable pour la pyronine des nucléoles, du cytoplasme et des mitochondries.

D'après nos essais d'extraction par les acides trichloracétique et chlorhydrique et de traitement par une solution de ribonucléase cristallisée, il semble que la pyroninophilie de ces cellules soit due à la présence d'acides nucléiques. Il s'agirait d'acide ribonucléique (Brachet 1947, Panijel) ou d'un mélange de celui-ci et d'acide désoxyribonucléique dépolymérisé (Kurnick), mais nous préférons le terme de pyroninophilie qui ne préjuge en rien des substances ainsi détectées et nous l'utiliserons dans la suite de cet exposé.

III. ETUDE DE L'OVAIRE

1. DESCRIPTION ET ANATOMIE DE L'ORGANE

A la dissection, l'ovaire se présente comme une frange blanche située à la base des muscles rétracteurs ventraux. Chez le mâle le testicule a la même disposition mais les digitations sont plus nombreuses et plus fines. Le volume de la gonade femelle varie avec l'état d'activité sexuelle de l'animal qui est moindre en hiver mais l'organe reste toujours très petit (moins d'un millimètre d'épaisseur), les ovocytes n'y subissant presque aucun accroissement. Sur des coupes transversales et sériées on constate que l'ovaire est rattaché à la base des muscles rétracteurs, près de leur insertion. Entre ces muscles, sous

le cordon nerveux, l'ovaire n'est pas interrompu mais se réduit et s'attache directement à la paroi du corps.

Une lame de tissu conjonctif d'apparence banale et en continuité avec le revêtement coelomique constitue à la frange ovarienne une sorte de mésentère qui la soutient sur toute sa longueur. Ce tissu conjonctif, qui revêt par ailleurs la gonade d'une mince enveloppe, se scinde dans sa masse en plusieurs bandes servant de support aux éléments germinaux. L'évolution de ces derniers s'effectue en direction du bord distal de l'organe d'où se détachent successivement des groupes d'ovocytes.

2. DIVERSES REGIONS DE L'OVAIRE ET CATEGORIES CELLULAIRES

Les éléments germinaux, gonies ou cellules primaires, ovogonies et ovocytes à différents stades d'évolution s'organisent par groupes entre les bandes de tissu conjonctif. Des cellules annexes sont dispersées entre les cellules reproductrices. On trouve, enfin, à raison de 2 ou 3 par coupe et généralement au contact du tissu conjonctif des éléments à contenu granulaire, très coloré par l'Altmann; leur rôle ne nous est pas connu mais ils présentent certains caractères des amoebocytes coelomiques (fig. 2 b).

Les stades d'évolution des cellules germinales sont répartis selon un gradient assez net pour permettre de diviser l'ovaire en 7 régions successives, 4 de celles-ci sont bien individualisées les autres étant plutôt des zones de transition (fig. 2 a).

Région I. (fig. 2 a et 2 b).

Sur la ligne de contact de l'ovaire et de la lame conjonctive qui le soutient on trouve des petits noyaux semblables à ceux de l'endothelium coelomique et contenant un nucléole (Champy-Altmann). On observe dans cette zone quelques mitoses, bien que les plaques équatoriales soient de petite taille on a pu, sur "squash" dénombrer dans ces noyaux diploïdes 20 chromosomes. Ces cellules sont vraisemblablement les *cellules primaires* qui sont à l'origine et des ovogonies et des cellules annexes.

Région II. (fig. 2 a, 2 b, 3 a, 3 b).

En dehors de quelques *cellules primaires* encore présentes dans cette région il apparaît ici 2 autres catégories cellulaires; des *ovogonies* en cours d'évolution, reconnaissables à leur grand noyau à chromosomes bien individualisés; et des *cellules annexes,* à noyau un peu plus grand que celui des cellules primaires et qui sont réparties à travers tout l'organe. On voit assez fréquement l'une de ces cellules annexes coiffant une ovogonie. Nous n'avons pas pu préciser, malheureusement, le mécanisme de ségrégation de ces 2 catégories cellulaires. Le pourcentage de cellules annexes et primaires dans la région II varie de 30 à 45 %.

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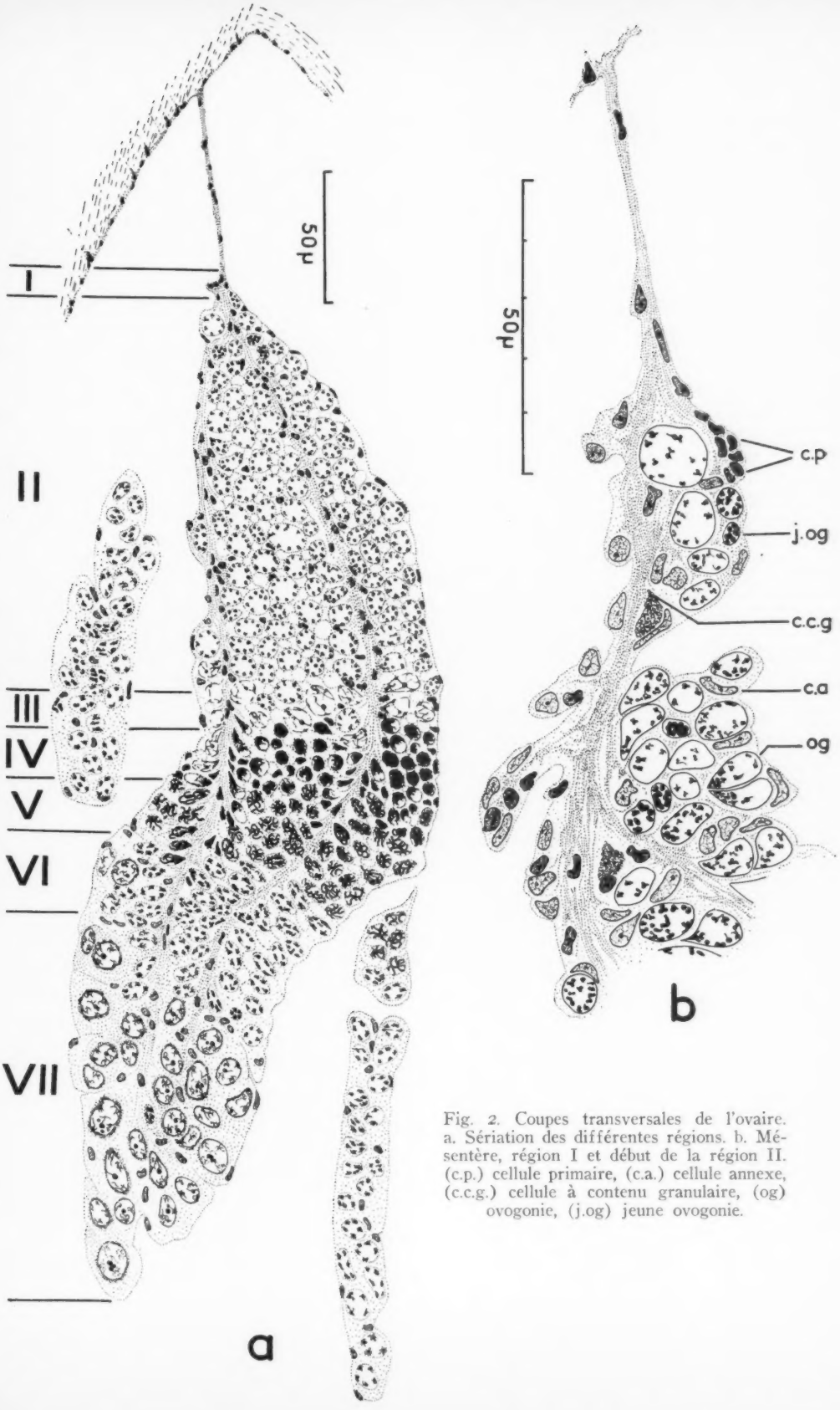

Fig. 2. Coupes transversales de l'ovaire. a. Sériation des différentes régions. b. Mésentère, région I et début de la région II. (c.p.) cellule primaire, (c.a.) cellule annexe, (c.c.g.) cellule à contenu granulaire, (og) ovogonie, (j.og) jeune ovogonie.

L'évolution des ovogonies paraît s'effectuer de la façon suivante: après une dernière division d'une cellule primaire les chromosomes restent individualisés à la télophase et se condensent en boules en nombre diploïde (20). On observe ensuite une déspiralisation très progressive des chromosomes qui ne sont pas répartis au hasard dans le noyau mais restent distribués près de la membrane nucléaire. Ces chromosomes adoptent finalement des formes assez complexes, vraisemblablement spécifiques. A ce stade un nucléole apparait. A la fin de cette période, au cours de laquelle la taille du noyau a augmenté progressivement, on assiste à une certaine polarisation de la cellule; les chromosomes se groupent à l'extrémité amincie du noyau.

Dans la série des coupes d'un même ovaire la région II peut présenter une importance très variable; en général, sur une coupe transversale, elle est très développée et active (mitoses) dans la mesure ou la région terminale (VII) est réduite et inversement. L'organe dans son ensemble n'évolue donc pas de façon synchrone, ses diverses parties fonctionnant indépendamment les unes des autres, se relayent pour produire continûment des ovocytes coelomiques. Nous avons d'ailleurs trouvé, dans le coelome, des ovocytes libres de taille minimum tout au long de l'année.

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Région III. (fig. 2 a, 3 b).

Les noyaux des ovogonies sont maintenant au stade *leptotène*. Les chromosomes se déspiralisent très fortement et se présentent sous forme de filaments longs et grèles plus ou moins contournés, souvent accolés à la membrane nucléaire. On voit un assez gros nucléole au contact de quelques éléments chromosomiques.

Les noyaux des cellules annexes paraissent subir aussi une certaine évolution, mais un stade leptotène y est difficilement perceptible.

La région III n'est jamais très développée ce qui semble indiquer une évolution assez rapide des cellules à son niveau.

Région IV. (fig 2 a, 3 c).

Les noyaux des ovogonies dans lesquels le nucléole a disparu entrent en phase *zygotène*. L'appariement des chromosomes est difficile à voir, même sur des "squash". On pourra distinguer cependant un premier stade avec appariement sans contraction de la masse chromosomique puis un synapsis très serré où une polarité se manifeste nettement. On constate une évolution synchrone des cellules annexes.

Les images nucléaires représentées ne semblent pas artificielles. La polarité des noyaux n'est pas due, par exemple, au sens de pénétration du fixateur car elle est toujours bien en rapport avec la position de l'axe conjonctif le plus proche, même en bordure de l'ovaire. Les noyaux des cellules annexes, à ce stade zygotène, sont cependant très fragiles; la compression de l'organe lors

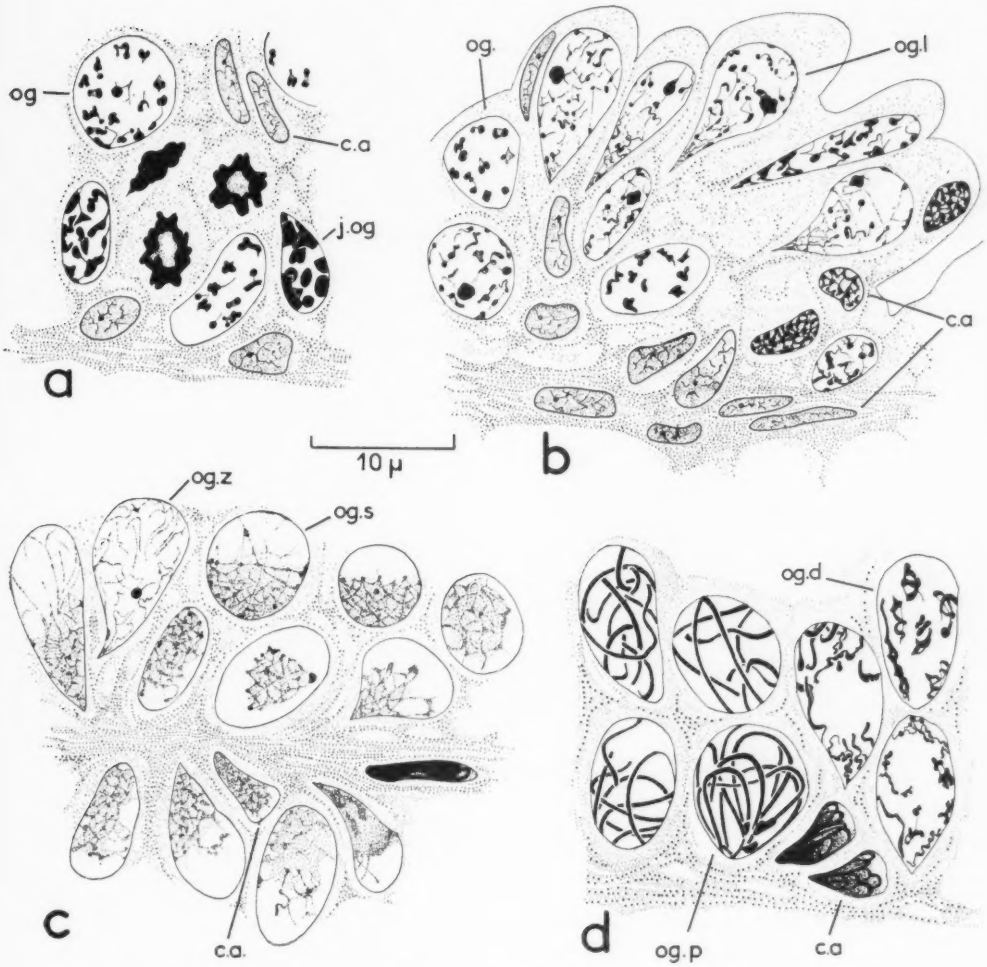

Fig. 3. Coupes transversales de l'ovaire. a. Détail de la région II. Mitoses. b. Détail des régions II et III. Stade leptotène. c. Détail de la région IV. Stade zygotène et synapsis. d. Détail des régions V et VI. Stades pachytène et diplotène. (c.a.) cellule annexe, (og) ovogonie, (j.og) jeune ovogonie, (og.l), (og.z), (og.s), (og.p), (og.d) stades leptotène, début de zygotène, synapsis, stades pachytène et diplotène dans les ovogonies.

de la dissection peut amener leur éclatement et faire apparaître une sorte de filament chromatique très allongé.

Région V. (fig. 2 a, 3 d).

Les ovogonies et les cellules annexes sont alors en phase *pachytène;* les chromosomes, groupés en bouquet, sont épaissis mais encore très allongés.

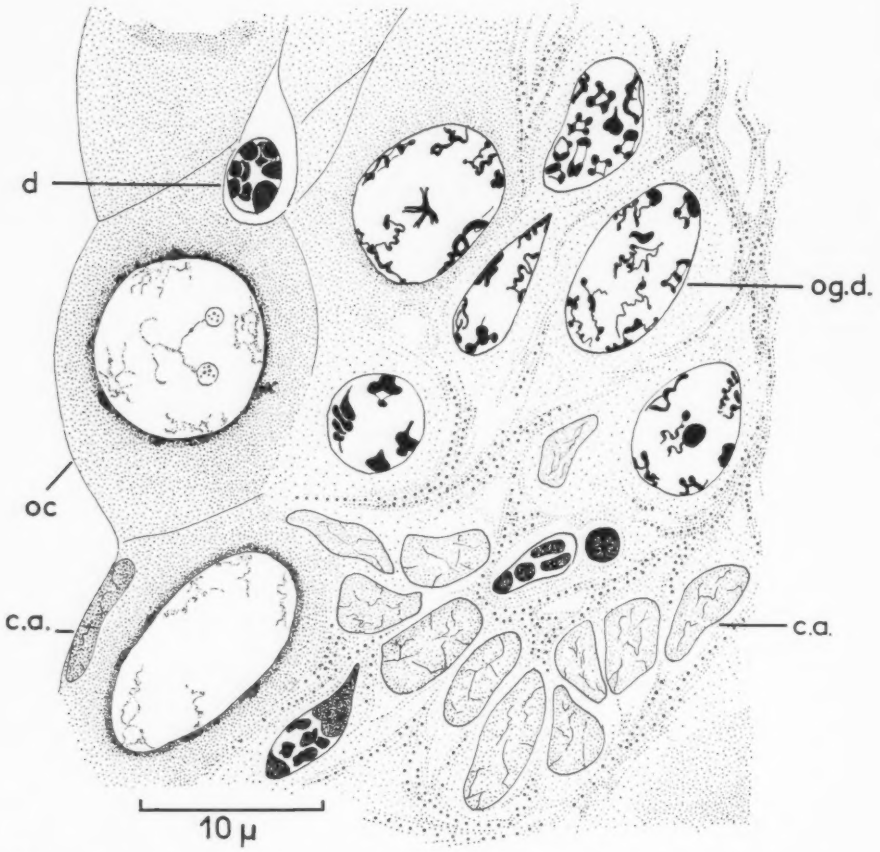

Fig. 4. Coupe transversale de l'ovaire. Détail des régions VI et VII. (og.d) ovogonie au stade diplotène, (oc) ovocyte, (c.a) cellule annexe, (d) cellule en voie de dégénérescence.

Région VI. (fig. 2 a, 3 d, et 4).

Les ovogonies sont maintenant au stade *diplotène*. Les chromosomes passent par une phase de forte spiralisation au cours de laquelle on peut observer des tétrades et différents types de chiasma.

Si les cellules annexes subissent la même évolution nucléaire, elles le font sans doute de façon assez brève car on voit réapparaître dans cette région de nombreux petits noyaux au repos.

Enfin on note un début d'accroissement cytoplasmique des ovogonies.

Région VII. (fig. 2 a, et 4).

C'est dans cette région que les ovogonies passent à l'état d'*ovocyte de premier ordre*. Les tétrades chromosomiques se distribuent à la périphérie du noyau et

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se déspiralisent progressivement tandis que leur colorabilité au Feulgen diminue. Des nucléoles apparaissent au niveau d'une figure chromosomique particulière : une tétrade en croix. Ils sont hétérogènes et contiennent des petits granules colorables par le Feulgen. Ce stade s'accompagne d'un net développement du volume nucléaire et cytoplasmique.

Parmi les ovocytes et les cellules annexes on trouve des noyaux de type aberrant qui pourraient être des stades d'involution. Certains contiennent 10 éléments chromosomiques structurés reconnaissables encore comme des groupes diploténiques. D'autres noyaux, très petits, renferment 10 masses chromosomiques plus ou moins distinctes et parfois tout à fait coalescentes, colorées en mauve et non plus en vert par le vert de méthyle pyronine. On observe souvent, autour de ces noyaux, une zone non colorable d'aspect vacuolaire. Ces éléments, d'aspect dégénératif, ont peut-être une origine mixte et seraient des cellules annexes et des ovogonies dans lesquelles la déspiralisation chromosomique suivant normalement le stade diplotène ne se produit pas. Mais leur origine pourrait aussi être exclusivement ovogoniale car nous avons trouvé dans la région VII un pourcentage de cellules annexes supérieur à celui observé dans la région II : il est ici de 40 à 50 % soit une augmentation de 5 à 10 % et l'on observe précisément 5 % environ de noyaux aberrants.

3. CYTOPLASME ET INCLUSIONS CYTOPLASMIQUES DES OVOGONIES ET DES OVOCYTES OVARIENS

Les ovogonies ont très peu de cytoplasme et la masse de l'ovaire, dans les régions I à V, paraît formée presque uniquement de noyaux et de tissu conjonctif (fig. 2 a). Cependant chez les ovogonies les plus précoces nous avons pu déceler par le vert janus des mitochondries accolées aux noyaux (fig. 5) ; aux phases leptotène ou zygotène elles sont groupées en petits amas dans la partie étroite des cellules.

Le cytoplasme commence à augmenter de volume dès que les noyaux ont atteint ou un peu dépassé le stade diplotène très spiralisé (région VI). La pyroninophilie cytoplasmique apparaît alors et se renforcera dans la région VII. A cet égard le cytoplasme des ovocytes prêts à se détacher de l'ovaire est colorable d'une façon homogène, si l'on excepte une zone périnucléaire beaucoup plus pyroninophile à inclusions très colorées. Cette zone ne montre aucune polarisation. L'Altmann ne colore pas les mitochondries des ovocytes de la région VII, mais le vert janus y montre des amas mitochondriaux assez nombreux, de grande taille et généralement beaucoup plus compacts que ceux des ovocytes libres de stade 1. On observe fréquemment, dans la région de contact de cellules voisines, deux amas mitochondriaux symétriques, nettement séparés par les membranes cellulaires (fig. 6).

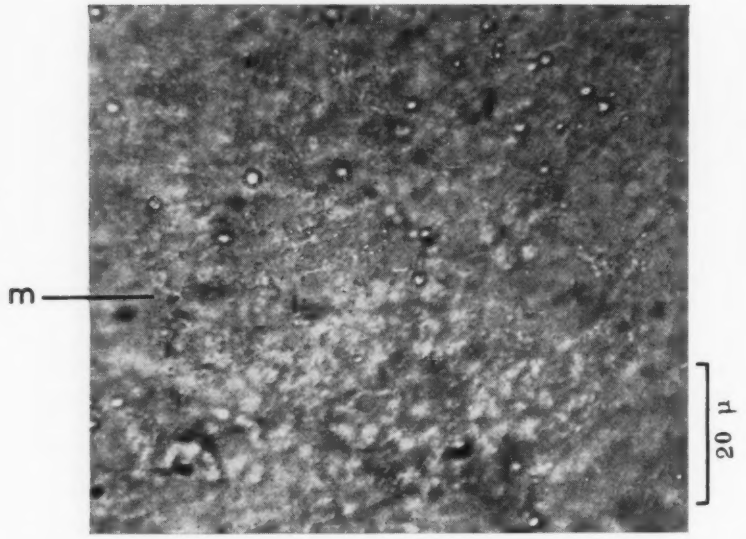

Fig. 5. Ovaire. Région basale. Préparation in toto. (m) mitochondries dans les ovogonies (vert janus).

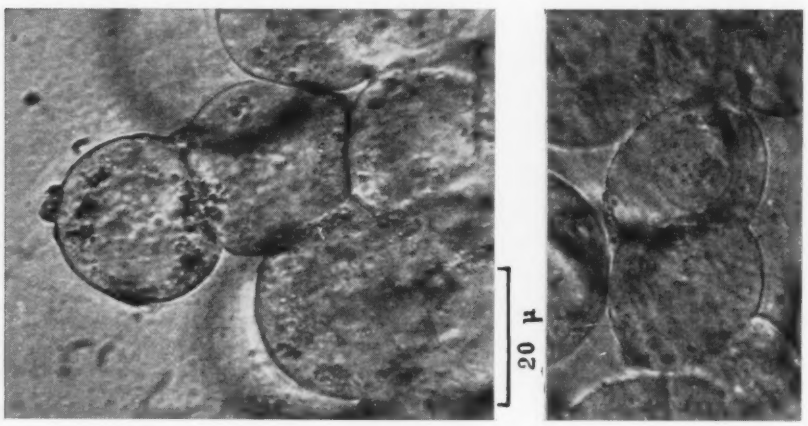

Fig. 6. Ovaire. Région distale. Amas mitochondriaux dans les ovocytes (vert janus).

4. RESUME

L'ovaire, bien que de dimensions réduites en hiver, est actif pendant toute l'année. C'est un organe assez simple qui, au fur et à mesure de l'évolution des cellules germinales, se fragmente par son bord libre. Il libère ainsi dans le coelome des groupes d'ovocytes ayant effectué leur prophase de meïose et commençant leur grand accroissement.

Dans l'organe les différents stades conduisant des gonies primaires aux ovo-

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cytes sont bien sériés. Des cellules primaires, à noyau d'aspect endothélial banal, donneraient par division les cellules annexes et les ovogonies. Ces ovogonies effectuent une prophase de meïose polarisée. Les cellules annexes subissent la même évolution nucléaire. Après le stade diplotène, dans le noyau des ovocytes, les chromosomes se déspiralisent, leur colorabilité diminue et des nucléoles apparaissent. Les ovocytes s'accroissent alors notablement; le cytoplasme plus abondant, devient très pyroninophile surtout dans la région périnucléaire. Dans les ovogonies comme dans les ovocytes on peut déceler des mitochondries. Un faible pourcentage d'ovocytes semble dégénérer après le passage au stade diplotène.

IV. ETUDE DES OVOCYTES LIBRES

L'étude cytologique de ces ovocytes a permis de diviser leur croissance en un certain nombre de phases distinctes. Bien que la détermination de ces phases ait été le but et le résultat de ce travail nous croyons préférable de tenir compte dès à présent de cette classification en étudiant les ovocytes stade par stade.

La croissance des ovocytes coelomiques peut se diviser en 3 stades principaux correspondant chacun, entre autres caractères, à l'élaboration prépondérante d'un certain type de réserve: polysaccharides, lipides puis plaquettes vitellines. Ces stades ont été dénommés: 1, 2, 3. Nous avons distingué également des stades transitoires: O, T, M. L'ordre de succession de ces différentes étapes est le suivant: O, 1, T, 2, 3, M.

STADE O (ou ovarien)

diamètre 20 à 30 μ.

Lorsque l'ovaire se fragmente sur son bord distal ce sont des groupes de plusieurs dizaines d'ovocytes qui sont libérés dans le coelome. A l'intérieur de ces amas les cellules, tassées les unes contre les autres, ne se retrouvent pas dans des conditions bien différentes par rapport au milieu ambiant: le liquide coelomique. Leurs caractères cytologiques sont ceux que nous avons décrits pour les ovocytes de la région VII de l'ovaire (fig. 4 et 6).

Dans ces amas sont dispersées des cellules annexes qui formeront aux ovocytes un follicule rudimentaire. Lorsque les ovocytes seront isolés les uns des autres, au stade 1, on constatera que cette enveloppe est formée de plusieurs cellules (3 à 5). Il faut admettre une multiplication des cellules annexes à l'intérieur des amas puisque dan l'ovaire on n'en comptait qu'une par ovocyte environ. Bien que ces éléments semblent comme les ovocytes subir une prophase de meïose, ils seraient donc susceptibles de se diviser peu après.

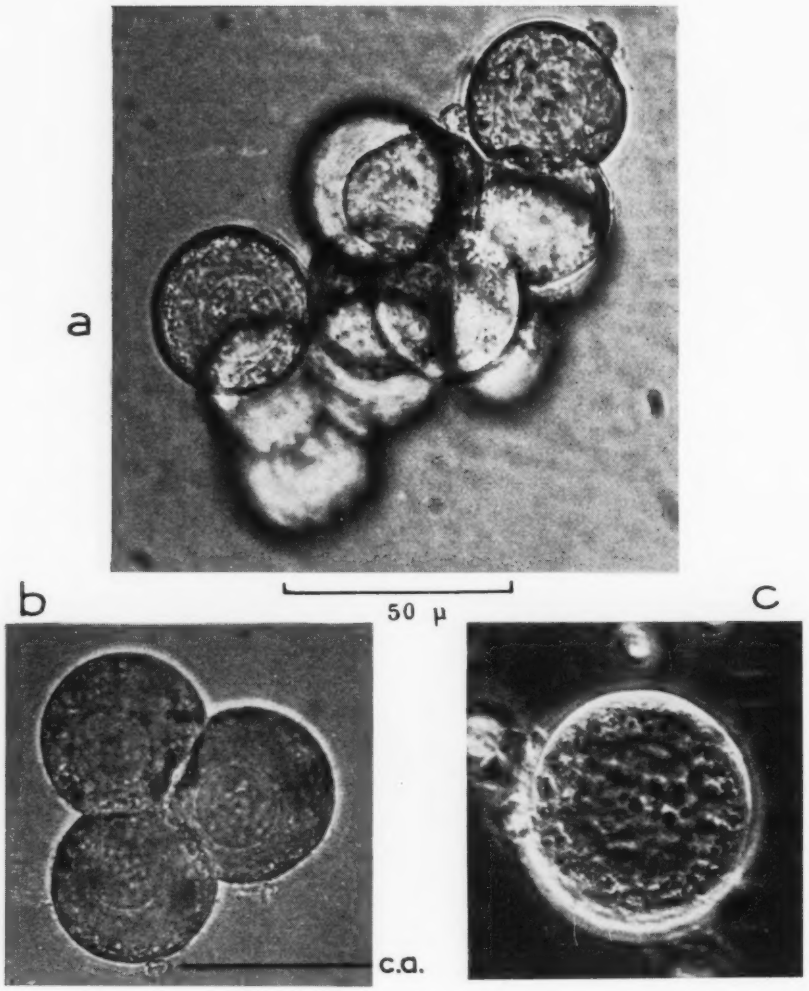

Fig. 7. Ovocytes libres. Stade 1. Sur le vivant. a. b. ovocytes en amas. Noter les mitochondries et les nucléoles, (c.a.) cellule annexe (noyau). c. ovocyte isolé. Noter les nucléoles et les nombreuses inclusions cytoplasmiques (contraste de phase).

STADE 1

diamètre : 30—35 μ ..... 60—65 μ
volume : 1,5 ..... $15 \cdot 10^{-5}$ μl.

Nous avons fixé le début de ce stade au moment où les ovocytes, s'étant peu à peu dégagés les uns des autres, ne se trouvent plus unis que de façon assez lâche par groupes d'une dizaine et adoptent une forme sphérique (fig. 7 a).

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C'est dans le cytoplasme de ces cellules que l'on distingue les premières traces de polysaccharide (fig. 8 d_1).

Sur le vivant les plus petits ovocytes ont un aspect clair, transparent qui devient jaunâtre dans les cellules de taille plus grande en raison de l'accumulation dans le cytoplasme de nombreuses inclusions granulaires (fig. 7 b, 7 c). Les ovocytes sont bien sphériques mais encore fragiles et déformables jusqu'à l'apparition d'une membrane vitelline mince à la fin du stade. Ils sont entourés individuellement d'une sorte de follicule rudimentaire formé par l'étalement en couche très mince de quelques cellules annexes dont on distingue seulement les petits noyaux qui font saillie à l'extérieur (fig. 7 b, 8 b_2).

Sur coupes la *pyroninophilie* du cytoplasme et de la zone périnucléaire avec ses inclusions est très forte (fig. 8 c) ; vers la fin du stade, cependant, cette zone diminue progressivement. Les *nucléoles*, également très pyroninophiles, se multiplient considérablement en restant accolés au centre du noyau (fig. 8 a_1, a_2) ; à la fin du stade I ils se répandent dans tout le volume nucléaire et perdent alors presque entièrement leur colorabilité à la pyronine (fig. 8 a_3, c_2).

Au cours du stade I le cytoplasme se charge progressivement en inclusions granulaires ; on l'observe sur coupes comme sur le vivant (fig. 7 b et c). Les *mitochondries* constituent des amas plus petits, mais plus nombreux que dans les ovocytes ovariens. Dispersés à la périphérie au début du stade I (fig. 7 b, 8 b_1), ces amas de granules se multiplient beaucoup jusqu'à gagner tout le cytoplasme (fig. 8 b_3). Les mitochondries sont colorables par l'Altmann, le noir soudan, le vert janus. Observés sur le vivant les amas mitochondriaux apparaissent formés de granules unis par une sorte de gelée plus consistante que le cytoplasme environnant. Bien que les granules ne soient pas en contact direct l'agitation intracellulaire affecte chaque amas dans son ensemble et montre que tous ses éléments sont solidaires.

Au début de ce stade, alors que les ovocytes sont encore groupés, on observe l'apparition, à la périphérie de la cellule, d'un *polysaccharide*, sous forme de grains très petits (fig. 8 d_1). Ces grains sont généralement disposés en cercles dont la dimension correspond à celle d'un amas mitochondrial. Ces inclusions se multiplient et s'accroissent jusqu'à atteindre 1/2 à 1 μ (fig. 8 d). Elles restent, semble-t-il, tojours localisées au voisinage immédiat du chondriome dont elles suivent le développement centripète au cours du stade I. Ce polysaccharide, probablement du galactogène, occupe finalement une fraction importante du volume cellulaire : près de 15 %.

Pendant le stade I, enfin, se différencie une *membrane vitelline* qui contiendrait des muco-protides (fig. 8 d_2).

Résumons les caractères principaux du stade I :

— Sur le vivant ; aspect clair devenant progressivement jaunâtre et granuleux dans les cellules plus grandes

— Forte pyroninophilie cytoplasmique

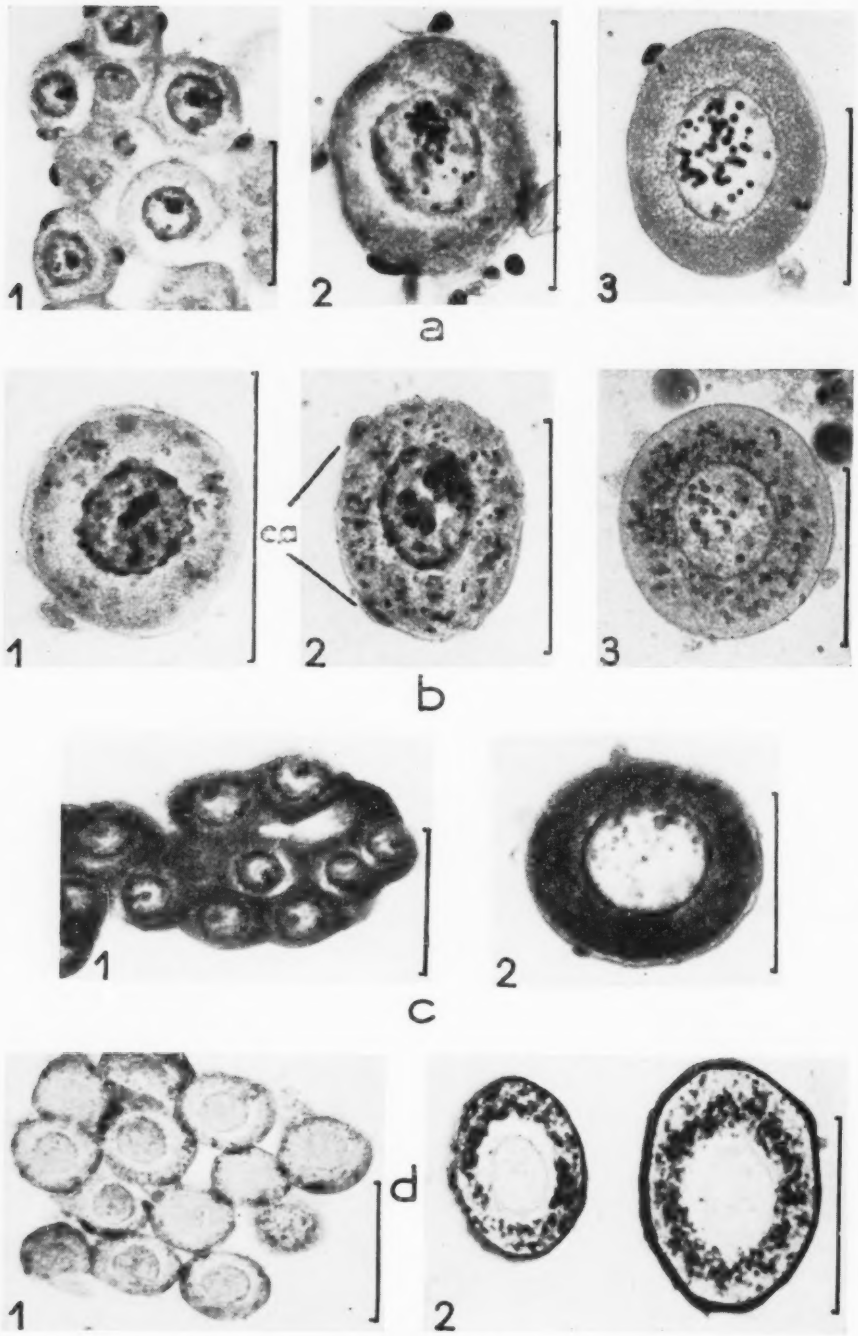

Fig. 8. Ovocytes libres. Stade 1. Coupes. a. Nucléoles (Helly-hématoxyline de Regaud). b. Chondriome (Champy-Altmann). c. Pyroninophilie (Helly-vert de méthyle pyronine). d. Polysaccharides (Helly-Mac Manus). (c.a.) cellules annexes, l'échelle correspond à 50 μ.

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— Multiplication des nucléoles
— Développement du chondriome
— Apparition d'un polysaccharide granulaire dans le cytoplasme
— Formation d'une membrane vitelline.

Enfin, tenant compte des résultats de notre étude biométrique, ajoutons que c'est à la fin du stade 1 que se bloque pendant l'hiver la croissance des ovocytes.

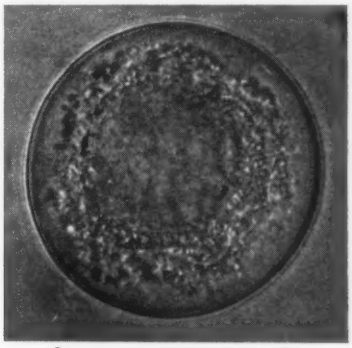

Fig. 9. Ovocyte libre. Stade T. Sur le vivant.

STADE T (ou transitoire)

diamètre : 60—70 μ
volume : 15.10^{-5} μl.

Le rassemblement des diverses inclusions autour du noyau est un des caractères les plus nets de ce stade ; il est bien visible *sur le vivant* (fig. 9).

Les *amas mitochondriaux* ne sont plus distincts mais forment au noyau une enveloppe épaisse et continue où l'on peut discerner un certain arrangement des granules par files radiaires (fig. 10 b et c). Le cytoplasme périphérique paraît vide de toute inclusion. Cet état de contraction du chondriome est tout à fait particulier au stade T. Dès le stade suivant (2) on verra les mitochondries refluer vers la périphérie de la cellule (fig. 12 b). Le *polysaccharide* granulaire ne suivrait que partiellement ce mouvement et reste dispersé en marge de l'enveloppe périnucléaire.

Un second caractère, également très net, de ce stade est la réduction brusque du nombre de *nucléoles* (fig. 18). Simultanément ces nucléoles deviennent plus gros, de forme assez irrégulière (fig. 10 a) et leur pyroninophilie, nulle à la fin du stade 1, redevient forte.

La *membrane vitelline* est mieux individualisée. Les *cellules annexes* se sont détachées de l'ovocyte et ont disparu.

On notera donc comme caractères principaux de ce stade :

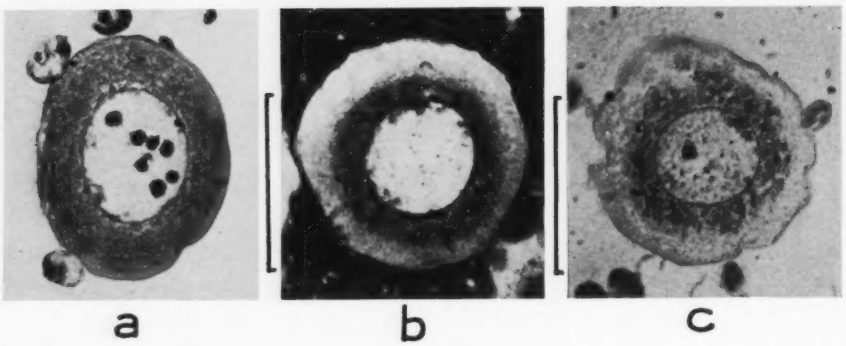

Fig. 10. Ovocytes libres. Stade T. Coupes. a. Nucléoles (Helly-hématoxyline de Regaud). b. Concentration des inclusions autour du noyau (Regaud-hém. de Regaud). c. Chondriome (Benoit-Altmann). L'échelle correspond à 50 μ.

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— La concentration autour du noyau de l'ensemble des inclusions cytoplasmiques et notamment des mitochondries
— La réduction du nombre de nucléoles
— La disparition des cellules annexes.

STADE 2

diamètre: 65—70 μ ..... 140—145 μ.
volume: 15 ..... $150 \cdot 10^{-5}$ μl.

Ce stade est surtout caractérisé par l'apparition et le développement considérable des *inclusions lipidiques,* celles-ci sont colorées, in vivo, et donnent aux cellules, aussitôt après le stade T, une teinte brune qui va en se renforçant au cours de la croissance des ovocytes. Dès son début la reconnaissance *sur le vivant* du stade 2 est donc très aisée. Les premières inclusions lipidiques sont reconnaissables dans l'enveloppe périnucléaire mitochondriale avant sa dispersion (fig. 12 d), puis continueront à se former dans toute la cellule jusqu'à occuper près de 40 % de son volume total.

La *pyroninophilie,* si elle persiste au niveau des mitochondries va en diminuant dans le cytoplasme au cours du stade 2 et il n'y a plus de zone périnucléaire caractérisée (fig. 12 a).

L'Altmann ne colore les *mitochondries* qu'au début du stade 2, à une période où, dans les ovocytes de 80 à 100 μ de diamètre, les mitochondries sont dispersées de façon homogène (fig. 12 b). Les résultats qu'apporte alors la pyronine sont peu nets en raison de la coloration générale du cytoplasme (fig. 12 a_1) mais dans les cellules d'une taille supérieure seule cette méthode nous permettra de mettre en évidence l'évolution du chondriome. Dans les ovocytes de 100 à 120 μ on observe ainsi de très gros amas mitochondriaux localisés à la périphérie

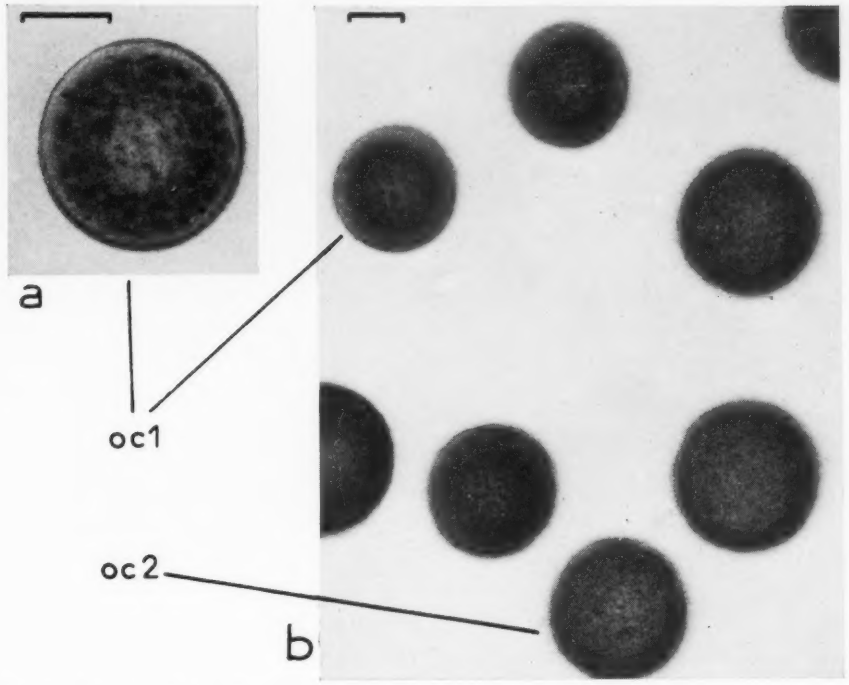

Fig. 11. Ovocytes libres. Stade 2. Sur le vivant. a. et b. coloration au vert janus. (oc. 1) ovocytes avec gros amas mitochondriaux périphériques, (oc. 2) ovocytes plus grands à chondriome dispersé. L'échelle correspond à 50 μ.

de la cellule (fig. 11 a et 12 a_2) ; on distingue également une zone mitochondriale moins nette autour du noyau et quelques amas intermédiaires. Cette phase très bien caractérisée est suivie, dans les ovocytes de 120 à 140 μ, d'une nouvelle dispersion du chondriome qui se fragmente en amas plus petits et répartis de façon homogène dans le cytoplasme (fig. 12 a_3). Ceci apparaît clairement sur le vivant après coloration au vert janus (fig. 11 a et b).

Le nombre de *nucléoles* augmente progressivement au cours du stade 2 passant de 10—20 à 30—40 (fig. 18). Ils sont, comme au stade T, de forme irrégulière du moins dans les ovocytes de 70 à 120 μ ; chaque nucléole est formé par l'agglomération autour d'un élément central, sphérique et pyroninophile, de nombreuses vésicules incolores (fig. 12 c). Dans les ovocytes de plus de 120 μ, c'est à dire au cours de la dernière partie du stade 2, les vésicules disparaissant, on note un retour des nucléoles au type régulièrement sphérique ; ils contiennent alors de nombreux granules Feulgen positifs. Puis la taille des nucléoles diminue et ils se distribuent, régulièrement espacés, contre la membrane nucléaire (fig. 14 d).

Le *polysaccharide* granulaire se disperse à travers le cytoplasme et commence,

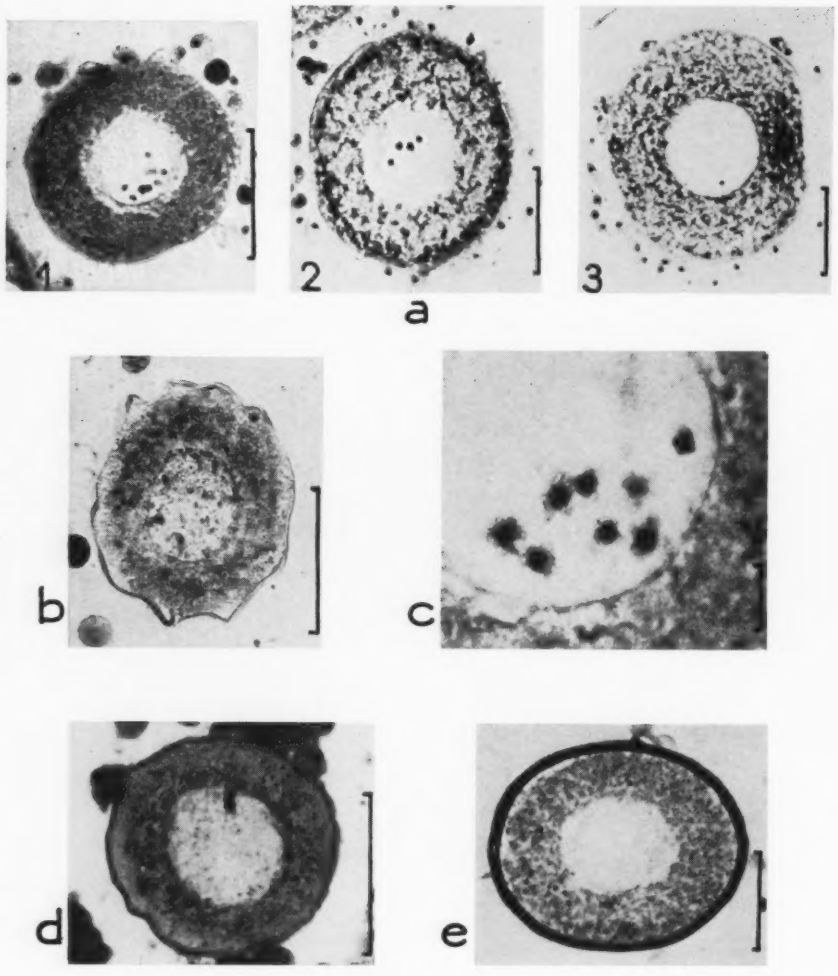

Fig. 12. Ovocytes libres. Stade 2. Coupes. a. Evolution de la pyroninophile (Helly-vert de méthyle pyronine). b. Chondriome au début du stade (Champy-Altmann). c. Nucléoles composites, 1ère moitié du stade (Helly-vert de méthyle pyronine). d. Apparition des inclusions lipidiques (Regaud-hém. de Regaud). e. Polysaccharides (Helly-Mac Manus). L'échelle correspond à 50 μ.

à la fin du stade, à migrer vers la périphérie de l'ovocyte et à constituer une couche de granules corticaux (fig. 12 e, 14 b).

Nous avons noté la présence, entre les inclusions lipidiques, d'un grand nombre de *petits éléments*, en grains, en bâtonnets, ou de forme irrégulière, colorables par le noir soudan. Le rouge neutre donne sur le vivant des aspects très comparables. Il s'agit peut-être d'éléments du vacuome ou en partie de mitochondries.

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La *membrane vitelline*, enfin, s'épaissit beaucoup au cours du stade 2, elle est percée de nombreux pores qui laissent passer de fins prolongements cytoplasmiques dont la longueur peut atteindre une vingtaine de microns (fig. 13).

Les caractéristiques principales du stade 2 sont donc:

— Sur le vivant; l'apparition et le renforcement d'une coloration brune
— La formation d'inclusions lipidiques
— Des modifications dans la localisation et la dimension des amas mitochondriaux
— La diminution de la pyroninophilie cytoplasmique
— Des transformations nucléolaires
— La dispersion du polysaccharide granulaire et sa migration vers la région corticale de l'ovocyte.

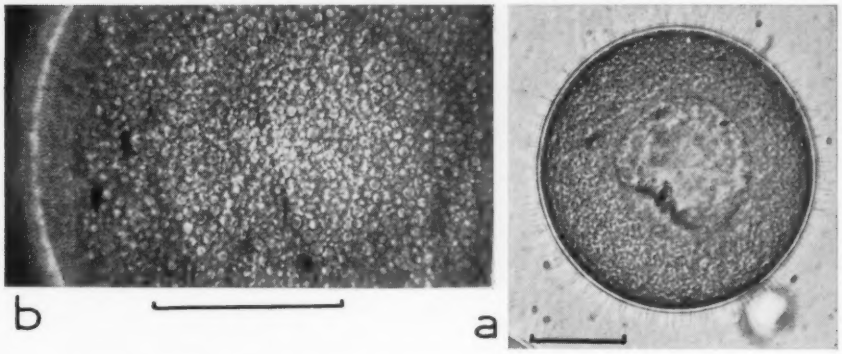

Fig. 13. Ovocytes libres. Stade 3. Sur le vivant. b. Coupe optique. Noter les inclusions lipidiques et à gauche les pores de la membrane vitelline.

STADE 3

diamètre: 140—145 μ ..... 160—170 μ
volume: 150 ..... $240 \cdot 10^{-5}$ μl.

Sur le vivant les caractères les plus nettement observables au cours de ce stade sont la forme dissymétrique du noyau (fig. 13) et l'éclaircissement progressif des ovocytes dont la couleur passe du brun foncé au jaune pâle. Mais l'on peut trouver, parfois, des ovocytes de grande taille dont la coloration est anormalement foncée car l'évolution des cellules, à l'intérieur de ce stade, n'est pas toujours exactement liée à leur accroissement en volume.

Sur coupe le caractère le plus net est sans doute la réapparition d'une *zone périnucléaire très pyroninophile* (fig. 14 a), d'aspect granulaire. Dans le reste du cytoplasme, dans les faibles espaces laissés entre les inclusions lipidiques, les petits *amas mitochondriaux* restent très pyroninophiles.

Le *noyau* prend une forme en haricot, sur la face concave la membrane nuc-

léaire présente un aspect déchiqueté (fig. 13, 14 a et b) et semble en liaison avec un gros *nucléole*. Les autres nucléoles, plus petits et moins nombreux qu'au stade précédent (fig. 18), sont de forme régulière, pyroninophiles et dispersés à la périphérie du noyau (fig 14 d).

Le phénomène le plus significatif est, au stade 3, la formation des *plaquettes vitellines*. Elles sont, malheureusement, d'une observation difficile parce que, peu nombreuses, dispersées parmi les inclusions lipidiques, elles n'apparaissent vraisemblablement que tout à la fin du stade 3. Bien que nous ayons réussi, dans un premier essai, à mettre des plaquettes en évidence par stratification intracellulaire, nous n'en avons retrouvé ultérieurement que dans 2 cas sur 8 dans des ovocytes de stade 3 provenant, cependant, de femelles en pleine période de reproduction (fig. 14 c).

D'autre part, sur coupes, nous n'avons pu les observer que dans un seul lot d'ovocytes fixés au mois de Mars. En effet, chez certains animaux, à cette époque de l'année on rencontre encore quelques gros ovocytes, parfois en dégénérescence, qui sont un reliquat de la dernière période de reproduction. Dans les cellules de stade 3 provenant de l'un de ces animaux nous avons remarqué une nette ségrégation des plaquettes vitellines et des inclusions lipidiques, accompagnée d'une polarisation de la cellule. Les plaquettes, en effet, sont rassemblées dans une zone de cytoplasme en croissant autour du noyau excentrique, cependant que les inclusions lipidiques sont rejetées à la périphérie (fig. 14 e). On remarque la présence de granules corticaux de grande taille (fig. 14 e_3).

Nous ne pensons pas que ces phénomènes constituent une étape normale du développement de l'ovocyte au cours du stade 3. Ils n'ont été observés qu'une seule fois et en dehors de la période de reproduction. C'est pourquoi nous considérons ces cellules comme des *ovocytes sur-développés*, mais la polarisation cytoplasmique qu'ils manifestent doit être rapprochée de celle que l'on remarque, de façon générale cette fois, dans le noyau des ovocytes de stade 3.

On ne trouve de *polysaccharide* granulaire qu'à la périphérie du cytoplasme sous forme d'une couche corticale (fig. 14 b). Une autre sorte de polysaccharide, apparentée par ses caractères histochimiques, au glycogène, est présente non sous forme figurée mais à l'état diffus, autour du noyau (fig. 14 b). On en relève également des traces au niveau de certains amas mitochondriaux et, par places, dans la région corticale de la cellule. Enfin, comme au stade précédent, le cytoplasme, entre les inclusions lipidiques, est chargé de nombreux *petits éléments* colorables par le noir soudan.

En résumé nous noterons au cours du stade 3 :

— sur le vivant, le changement de coloration des cellules passant du brun au jaune clair, la polarisation du noyau
— L'apparition des plaquettes vitellines

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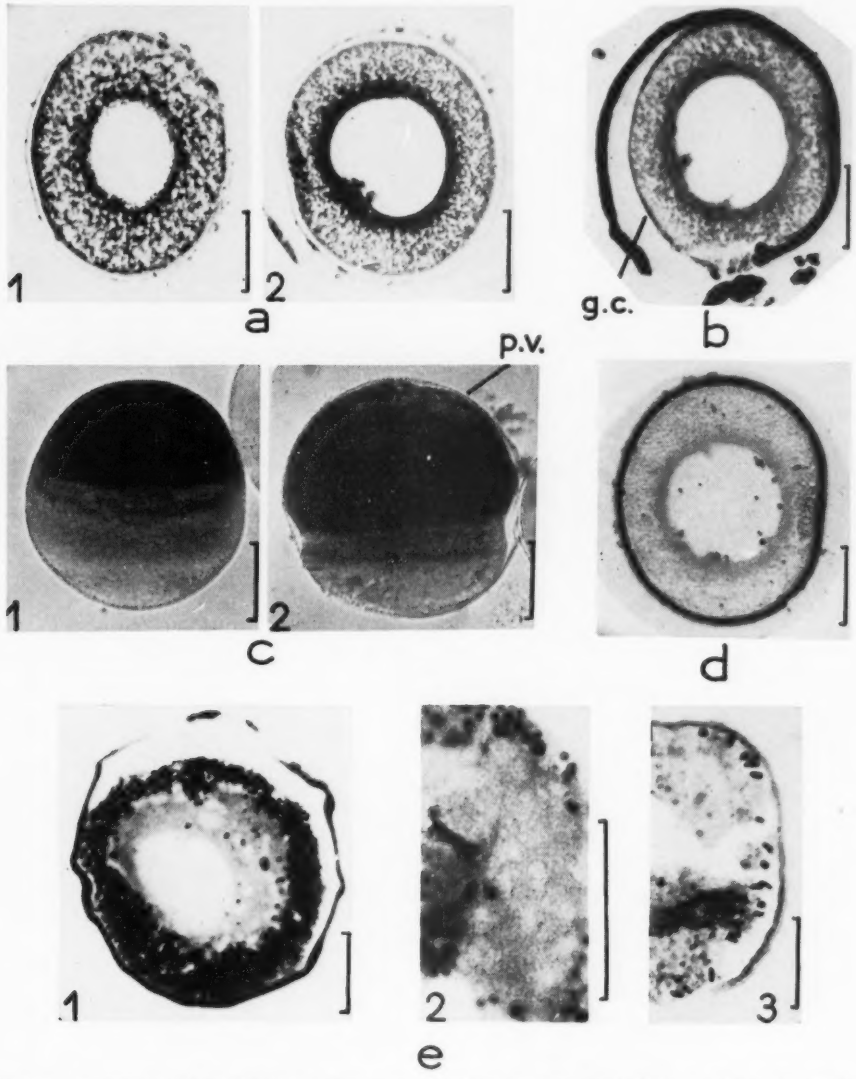

Fig. 14. Ovocytes libres. Stade 3. Coupes et fixations. a. Pyroninophilie, a_1 début du stade (Helly-vert de méthyle pyronine). b. Polysaccharides (Helly-Mac Manus), (g.c.) granules corticaux. c. Plaquettes vitellines. c_1 ovocyte au début du stade 3 sans plaquettes, c_2 ovocyte plus agé, plaquettes (p.v.) au pôle centripète. (fixation et coloration : ac. osmique). d. Nucléoles périphériques (Helly-hém. de Regaud). e. Ovocytes sur-développés (Champy-Altmann). e_1 : Inclusions lipidiques périphériques. e_2 : Région cytoplasmique périnucléaire contenant les plaquettes vitellines. e_3 : éléments corticaux. L'échelle correspond à 50 μ.

— La forte pyroninophilie de la zone périnucléaire
— La couche de granules corticaux (galactogène ?) et la formation d'un nouveau polysaccharide (glycogène ?).

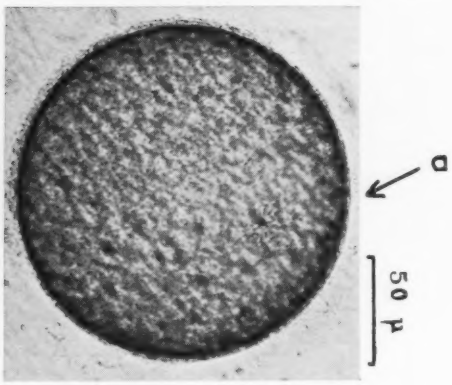

Fig. 15. Ovocyte libre. Stade M. Sur le vivant. (a) asters.

STADE M (Maturation)

diamètre : 160—170 μ.
volume : $240 \cdot 10^{-5}$ μl.

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Ce dernier stade correspond à la *maturation* de l'ovocyte. La membrane nucléaire s'efface ; il se forme 2 asters excentriques et de petite dimension (fig. 15) ; les chromosomes en nombre haploïde se groupent sur le fuseau en position métaphasique. Ce phénomène très rapide peut être déclenché dans certains ovocytes de stade 3 jaune clair par lavage et traitement à l'eau de mer. On l'observe, chez un animal prêt à pondre, à la fois dans le coelome et dans les néphridies par l'intermédiaire desquelles se fait l'évacuation.

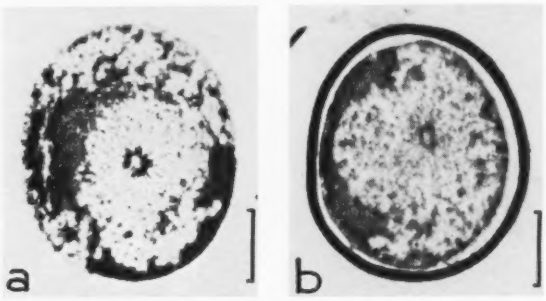

Fig. 16. Ovocytes libres. Stade M. Coupes. a. Pyroninophilie (Helly-vert de méthyle pyronine). b. Polysaccharides (Helly-Mac Manus). Disparition des granules corticaux, décollement de la membrane vitelline. L'echelle correspond à 50 μ.

Sur coupes l'organisation intracellulaire apparaît assez bouleversée. De grandes *plages pyroninophiles*, situées à une certaine distance du fuseau semblent correspondre à la zone périnucléaire du stade 3 (fig. 16 a). On y met en évidence des *mitochondries* et le *polysaccharide* diffus du stade précédent (fig.

16 b). Les inclusions lipidiques sont à la périphérie de la cellule mais on en trouve également à proximité du fuseau. On remarque la disparition des granules corticaux et le décollement de la membrane vitelline qui reste reliée à la membrane cytoplasmique par de fins tractus (fig. 16 b).

DONNEES NUMERIQUES

Les nucléoles et les mitochondries nous ont paru assez bien définis cytologiquement pour tenter une étude de leur évolution numérique au cours de la croissance des ovocytes.

Pour dénombrer les mitochondries on a tenu compte des granules individuellement et non des amas dont la taille, nous l'avons vu, varie d'un stade à l'autre. La coloration au vert janus n'a malheureusement pas pu être utilisée. Nous avons essayé de colorer les mitochondries par cette méthode et de les compter dans un volume de cytoplasme limité par un élément d'oculaire quadrillé, la numération devant aller jusqu'au noyau puisque la répartition des mitochondries n'est pas homogène à travers la cellule ni constante pendant la croissance. Or la coloration se développe assez mal en profondeur et la présence d'inclusions lipidiques, nuisant à la netteté, rend le comptage très imprécis au delà d'une région proche de la périphérie de la cellule.

Nous avons dû, par conséquent, opérer sur des coupes colorées soit par le vert de méthyle pyronine soit par l'Altmann (fixation au Helly ou au Champy, coupes à 6 μ). Les mitochondries ont été dénombrées dans une tranche de cytoplasme allant du noyau à la membrane vitelline et limitée par 2 traits parallèles du micromètre oculaire. On déterminait ainsi un volume d'une largeur de 15 μ, d'une épaisseur de 6 μ correspondant à celle de la coupe mais dont la longueur ne peut se mesurer directement car il faut tenir compte des contractions qu'entraîne la fixation. Celle du cytoplasme paraît faible, ainsi que l'on peut en juger en comparant les dimensions du stade T, par exemple, sur coupes et sur le vivant. Par contre la contraction du noyau n'est pas négligeable et pour la compenser nous n'avons tenu compte, sur les coupes, que du diamètre total de la cellule étudiée, nous reportant ensuite à un abaque, obtenu sur le vivant, donnant la distance noyau—membrane vitelline en fonction du diamètre. Les résultats sont donnés en nombre de granules par unité de volume soit 10^{-5} μl. Des numérations répétées dans une même zone ou effectuées dans plusieurs régions d'une même cellule homogène font apparaître une erreur relative de 20 % au plus.

Nous avons pris un certain nombre de précautions d'ordre statistique au cours de ce travail; par exemple le dénombrement dans 4 zones différentes pour chaque cellule, mais l'on doit admettre que les bases en sont assez criticables. Il nous a fallu utiliser 2 colorations différentes car l'une, l'Altmann ne

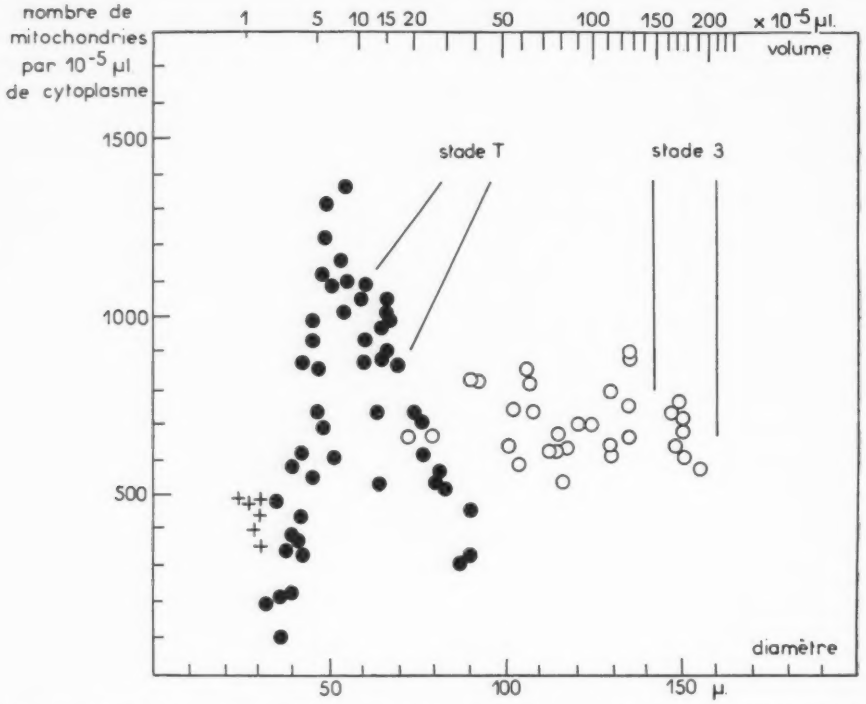

Fig. 17. Nombre de mitochondries par unité de volume de cytoplasme. + colorées par le vert janus. ● par l'Altmann. ○ par la pyronine.

colore les mitochondries qu'aux stades 1 et T et l'autre, le vert de méthyle pyronine qu'aux stades 2 et 3. Il n'est guère possible, dans ces conditions, de comparer les résultats obtenus au cours de ces 2 phases de la croissance. On reconnaîtra seulement la multiplication des mitochondries au cours du stade 1 et la stabilité de leur nombre pendant le stade 2. Nous indiquons également le résultat de quelques numérations effectuées sur des ovocytes de stade 0; dans ces cellules, les mitochondries après coloration au vert janus se comptent aisément étant donné l'absence d'autres inclusions (fig. 17).

La numération des nucléoles a été effectuée sur des coupes sériées colorées à l'hématoxyline de Regaud (fixation au Helly, au Regaud, coupes à 8 μ) (fig. 18). Des observations sur le vivant, en contraste de phase, ont donné des résultats très comparables pour les petits ovocytes (fig. 7 c); à partir du stade T l'accumulation d'inclusions diverses dans le cytoplasme ne permet plus une observation claire du noyau.

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STADES DE CROISSANCE DES OVOCYTES

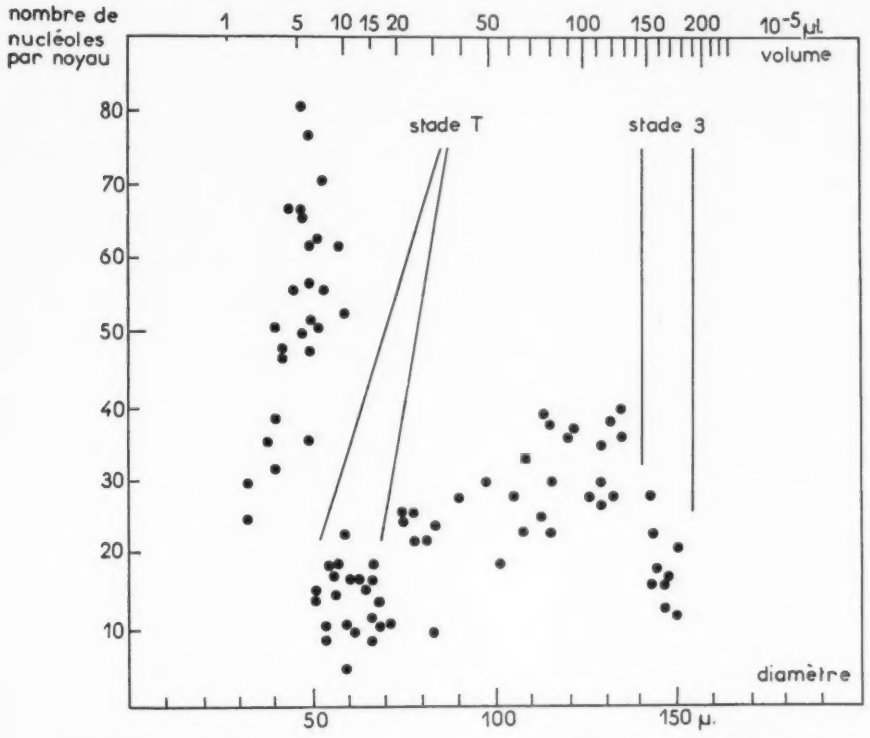
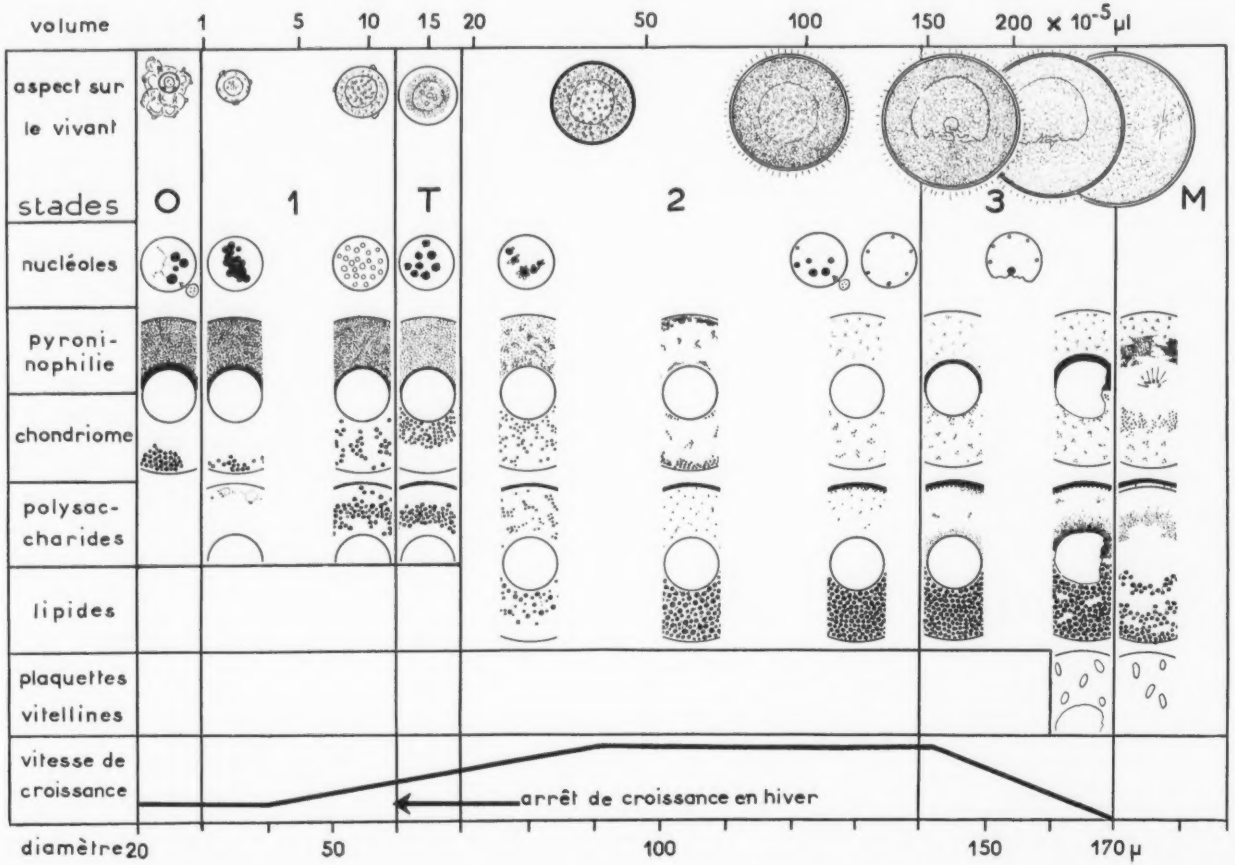


Fig. 18. Nombre de nucléoles par noyau, colorés par l'hématoxyline de Regaud.

V. CONCLUSION

Nous avons étudié l'ovaire de Phascolosoma vulgare afin de définir le stade de libération des ovocytes. On a constaté que l'organe est en état d'activité toute l'année et on a suivi la transformation des cellules primaires en ovocytes et en cellules annexes. Les ovogonies subissent dans la gonade une prophase de meïose menée jusqu'à un stade diplotène assez avancé. Puis les chromosomes se déspiralisent et des nucléoles apparaissent, ces phénomènes allant de pair avec un début d'accroissement cellulaire. Le cytoplasme, alors plus abondant, est très pyroninophile surtout dans une zone périnucléaire et renferme un certain nombre d'amas de granules mitochondriaux. C'est alors que les ovocytes se détachent de l'ovaire et vont poursuivre dans le coelome leur croissance à peine ébauchée.

L'étude de ces ovocytes coelomiques nous a permis de distinguer au cours de leur évolution un certain nombre de stades bien définis, caractérisés par


volume
1
5
10
15
20
50
100
150
200 x 10⁻⁵ µl
aspect sur le vivant
stades
O
1
T
2
3
M
nucléoles
pyroni-nophilie
chondriome
polysac-charides
lipides
plaquettes vitellines
vitesse de croissance
arrêt de croissance en hiver
diamètre
20
50
100
150
170 µ

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des changements morphologiques et histochimiques. Un tableau groupe l'ensemble de ces observations et quelques résultats d'une étude biométrique des ovocytes libres publiée parallèlement.

Nous insisterons ici sur le synchronisme rigoureux entre la croissance volumétrique d'une cellule et son évolution morphologique et physiologique. La confrontation des résultats obtenus par différentes méthodes met ce phénomène clairement en évidence. On constate, par exemple, que le passage d'un stade cytologique au suivant s'accompagne à la fois d'une modification dans la vitesse de croissance et d'une variation dans l'intensité respiratoire. Il s'ensuit que, de la seule connaissance du diamètre d'un ovocyte, on peut déduire, en fonction des études effectuées précédemment, ses autres caractéristiques. C'est là, nous semble-t-il, l'un des aspects intéressants de ce matériel.

Nous reprendrons l'analyse de ces résultats et les commenterons dans une publication ultérieure concernant le métabolisme respiratoire des ovocytes. Nous ferons alors les rapprochements nécessaires, stade par stade, entre les différents aspects que présente la croissance de ces cellules.

Je tiens à exprimer ma reconnaissance à M. le Professeur Fromageot pour l'interêt si bienveillant qu'il a porté à l'ensemble de mon travail. Je remercie particulièrement, parmi tous ceux qui m'ont prodigué leur appui ou leurs conseils, MM. les Professeurs Fauré-Frémiet, Drach, et Bocquet (ainsi que M. le Professeur P. E. Lindahl qui a bien voulu prendre soin de la publication de ce travail). Je remercie M. le Professeur Teissier de son hospitalité à la Station Biologique de Roscoff.

AUTEURS CITES:

ANDREWS, A., 1889. The reproductive organs of Phascolosoma gouldii. Zool. Anz. *12*, 140—142.

BRACHET, J., 1937. Remarques sur la formation de l'acide thymonucléique pendant le développement des oeufs à synthèses partielles. Arch. Biol. *48*, 529—548.

BRACHET, J., 1947. Embryologie chimique. Paris, Liège.

COOPERSTEIN, S. J., LAZAROW, A. & PATTERSON, J. W. 1953. Studies on the mecanism of Janus green B staining of mitochondria. Exp. Cell Res. *5*, 56—97.

CUENOT, L., 1900. Le Phascolosome in Zoologie descriptive. O. Doin, Paris.

GEROULD, J. H., 1906. The development of Phascolosoma. Zool. Jahr. *23*, 77—162.

GLICK, D., 1949. Techniques of Histo- and Cytochemistry. New-York.

GONSE, P. H., 1953. L'ovogenèse chez Phascolosoma vulgare. (note préliminaire) C. R. Ac. Sc. *236*, 528—530.

GONSE, P. H., 1954. Respiration de l'ovocyte au cours de sa croissance. C. R. Ac. Sc. *238*, 2350—2352.

GONSE, P. H., 1955. Recherches sur la physiologie de l'ovogenèse chez Phascolosoma vulgare. Exp. Cell Res. *8*, 550—553.

GRAINGER, J. N. R. & SHILLITOE, A. J., 1952 Histochemical observations on galactogen. Stain Technology *27*, 81—85.

HERUBEL, M. A., 1908. Recherches sur les Sipunculides. Paris.

KURNICK, N. B. 1955. Histochemistry of nucleic acids. Intern. Rev. Cytology *4*, 221—268.

LANGERON, M., 1942. Précis de microscopie. Paris.

LILLIE, R. D., 1951. Histochemical comparison of the Casella, Bauer, and periodic acid oxidation — Schiff leucofuschin technics. Stain technology *26*, 123—136.

LINEWEAWER, H. & JANSEN, E. F., 1951. Pectic enzymes. Advances in enzymology. *XI*, 267—295.

LISON, L., 1936. Histochimie animale. Paris.

PANIJEL, J., 1951. Les problèmes de l'Histochimie et la Biologie cellulaire. Paris.

SELENKA, E., 1875. Eifurchung und Larven bildung von Phascolosoma elongatum Kef. Zeit. Wiss. Zool. *25*, 442—450.

SERRA, J. A., 1946. Histochemical tests for Protein and amino-acids, the characterisation of basic proteins. Stain technology *21*, 5—18.

THOMAS, J. A., 1932. Recherches cytologiques et expérimentales sur les vésicules énigmatiques et les urnes du Siponcle. Arch. Zool. Exp. et Gén. *73*, 22—40.

VOLKONSKY, M. & MARCOU, J., 1933. Les lignées leucocytaires des Sipunculides. Arch. Anat. micro. *29*, 245—260.

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L'OVOGENESE CHEZ PHASCOLOSOMA VULGARE
II. RECHERCHES BIOMETRIQUES SUR LES OVOCYTES

PAR

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INTRODUCTION

Chez Phascolosoma vulgare (Blainv.) les femelles ne parviennent à maturité sexuelle qu'au cours d'une période limitée allant de Juin à Septembre. Ce caractère saisonnier de l'activité reproductrice peut être dû à deux phénomènes: soit à un arrêt hivernal de l'activité ovarienne au niveau, par exemple, de la multiplication des gonies primaires; soit à un blocage de la croissance survenant chez les ovocytes coelomiques. Or nous avons remarqué que l'ovaire est fonctionnel toute l'année et libère continuellement des petits ovocytes dans la cavité générale. C'est donc la seconde hypothèse qui a retenu notre attention et nous avons cherché à préciser par une analyse biométrique à quelle étape de leur croissance les ovocytes libres sont sensibles aux facteurs saisonniers. Nous avons pu reconnaître ce stade d'une part et mettre en évidence, d'autre part, des variations de la vitesse de croissance de ces cellules.

TECHNIQUES

On prélève à la seringue environ 100 µl. de liquide coelomique qui sont ensuite dilués à l'eau de mer dans une salière. La suspension est aussitôt agitée afin d'empêcher l'agglutination d'un mélange d'ovocytes, d'hématies et de leucocytes au fond du récipient. On agitera à nouveau pour éviter les effets d'une décantation différentielle au moment de prélever à la pipette une certaine quantité de cette suspension que l'on dispose dans des lames creuses sous lamelle. Ces lames dont la cuvette est peu profonde permettent l'observation à sec avec un objectif 40 (grossissement total 640). On mesure alors avec un micromètre oculaire le diamètre de 250 ou 500 ovocytes suivant l'étalement des valeurs. La lame est balayée entièrement mais sans recoupements et en tenant compte de tous les ovocytes passant dans le champ. Ces mesures ont été géné-

ralement effectuées de semaine en semaine sur des animaux isolés en élevage pendant 1 mois à une température voisine de celle du milieu naturel. Cependant afin d'éliminer l'influence éventuelle des conditions d'élevage, des piqûres et des prélèvements répétés de liquide coelomique, de nombreuses observations ont été faites sur des animaux fraîchement récoltés et dont on prélevait le liquide coelomique en totalité. D'autre part, à titre de contrôle, des séries de 500 mesures ont été effectuées en 2 fois sur 2 prélèvements successifs de liquide coelomique d'un même animal.

Les résultats bruts, obtenus en graduations du micromètre oculaire, ont été groupés en histogrammes à classes de 3 graduations. La conversion de ces résultats en microns amenait des décimales rendant ainsi le regroupement par classes difficile. Dans les diagrammes que nous donnons ici nous avons donc conservé en abcisse les classes formées à partir des graduations micromètriques. Afin de permettre la comparaison avec d'autres résultats nous indiquons toutefois quelques valeurs de diamètre en microns, correspondant au milieu des classes dont chacune couvre 5,7 μ. Les courbes ont été obtenues en joignant les milieux des sommets des classes. L'erreur sur la mesure du diamètre est de 5 %. Précisons enfin que ces résultats ne concernent que les ovocytes isolés, c'est-à-dire des cellules parfaitement sphériques, et que 30 animaux ont été ainsi analysés au cours d'une année.

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ANIMAUX D'HIVER

Pendant les mois de Décembre, Janvier et Février nous avons observé une forte prédominance d'animaux ne contenant que des petits ovocytes. Certains renferment aussi des ovocytes de grande taille, souvent en dégénérescence, mais on n'observe aucune cellule de dimension intermédiaire.

La classe la plus riche en petits ovocytes est située le plus souvent à 39,9 μ, mais parfois à 45,6 μ ou 34,2 μ. Comme nous n'avons effectué aucune mesure d'ovocytes en amas la plus faible de ces valeurs est le diamètre moyen des plus petits ovocytes libres et isolés, à la largeur de la classe près soit : 34,2 $\pm$ 2,9 μ. Les cloches manifestent une nette tendance à l'asymétrie lorsque leur mode se situe à 39,9 μ et surtout à 45,6 μ ; on observe alors une inflexion sur leur droite. Chez ces animaux à courbe unimodale, l'étalement de la cloche est faible ; tous ceux que nous avons examinés ne renfermaient que des ovocytes de diamètre inférieur à 70,3 μ, si l'on fait abstraction des gros ovocytes en dégénérescence. La moyenne des diamètres maxima observés chez 12 animaux est 60,8 μ (extrêmes : 43,7—70,3 μ). Nous avons d'autre part analysé des animaux de semaine en semaine (fig. 1) et constaté qu'il n'y a pas de différence sensible entre les histogrammes obtenus au cours d'une période de 3 semaines.

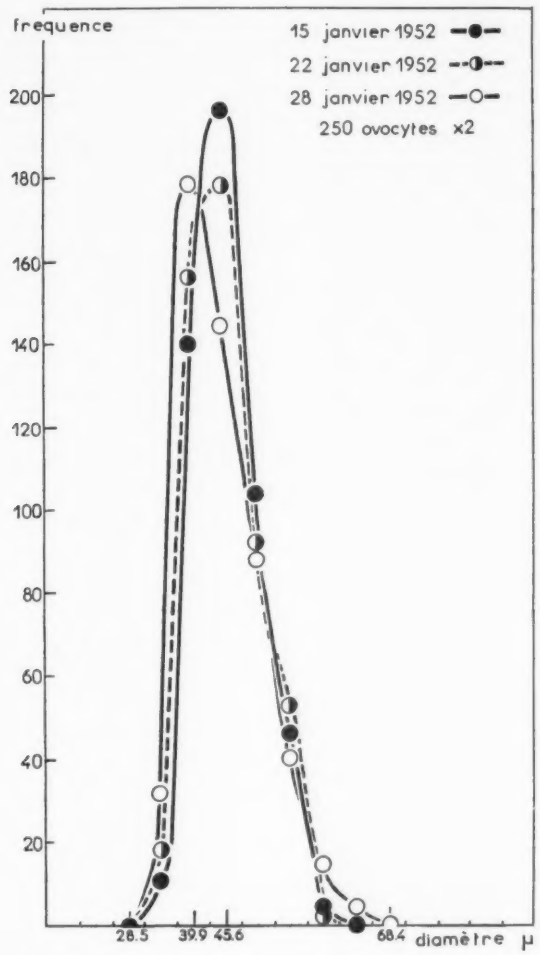

Fig. 1. Fréquence des tailles de 250 ovocytes coelomiques en hiver. 3 courbes successives obtenues chez un même animal.

Il semble, d'après ces résultats, que les ovocytes détachés en hiver ne s'accroissent que dans une faible mesure; après s'être isolés les uns des autres ils atteindraient lentement une taille limite (diamètre 60 μ).

ANIMAUX DE PRINTEMPS

Dès le mois de Février mais surtout en Mars et en Avril on observera des histogrammes très différents, d'allure bimodale, avec un plus grand étalement vers les tailles moyennes.

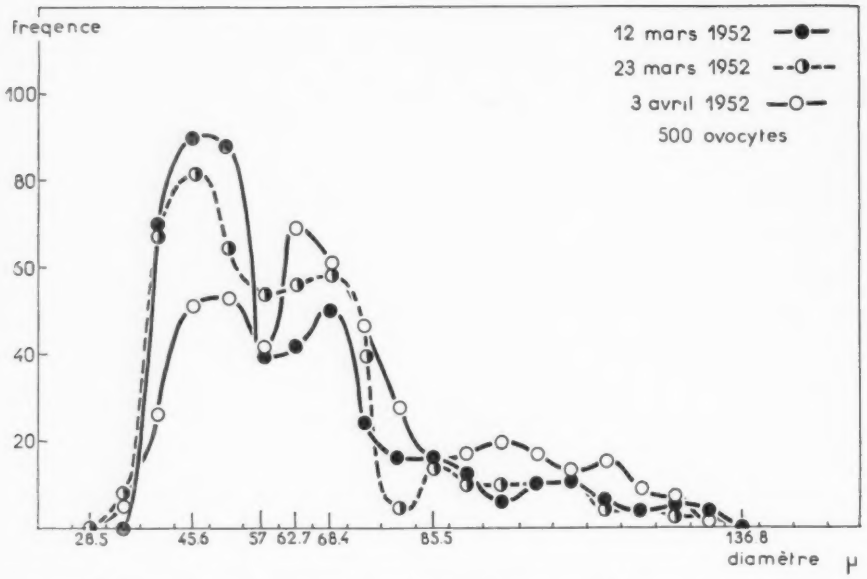

Fig. 2. Fréquence des tailles de 500 ovocytes coelomiques au printemps. 3 courbes successives obtenues chez un même animal.

On trouve toujours un premier maximum pour 45,6 μ, mais également un second pour un diamètre moyen de 66,1 μ (extrèmes: 62,7—68,4 μ). La position de ces deux maxima est constante, qu'il s'agisse d'animaux pris au hasard ou d'animaux conservés en élevage et examinés à intervalles de temps successifs. Dans ce dernier cas on voit, contrairement à ce que nous avions observé en hiver, une évolution se dessiner dans un intervalle de 3 semaines (fig. 2). La décroissance de la première cloche est en corrélation avec l'accroissement de la seconde. L'abaissement très prononcé ici de la première cloche est vraisemblablement dû à ce que le tranfert d'une grande partie des cellules qui la composaient vers la cloche suivante dépasse en importance l'afflux régulier des petits ovocytes détachés de l'ovaire.

On pourrait en conclure que les ovocytes de diamètre supérieur à 46 μ ont une croissance plus rapide et qu' à la fin de l'hiver, après une période d'arrêt plus ou moins longue, leur évolution reprend.

ANIMAUX D'ETE ET D'AUTOMNE

En été, aux mois de Juin, Juillet et Août on constate la persistance d'un mode pour les petites tailles et l'apparition d'une cloche correspondant aux plus gros ovocytes; les fréquences intermédiaires sont toujours faibles. On peut ren-

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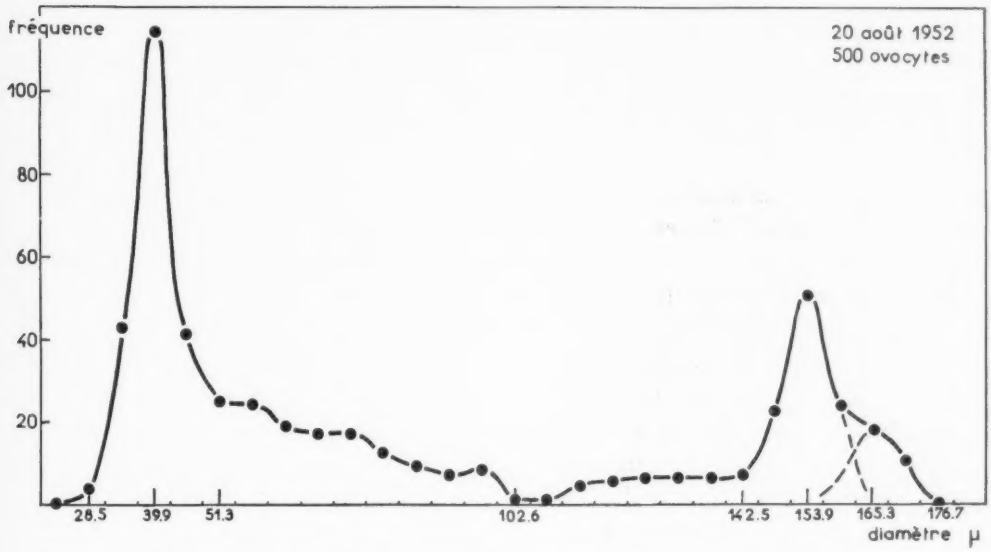

Fig. 3. Courbe de fréquence des tailles de 500 ovocytes coelomiques en été.

contrer des animaux de ce type, mais en nombre restreint, en Mai et en Septembre.

Le maximum de la deuxième cloche qui se situe à 153,9 μ ne paraît pas correspondre à l'arrêt final de la croissance. En effet les ovocytes mûrs, prêts à être pondus, ont 160 à 165 μ de diamètre. Il paraît donc légitime de décomposer cette cloche en une première population d'ovocytes à diamètre moyen de 153,9 μ (étalement: 146,3 à 161,5 μ) et une seconde à 165,3 μ (étalement: 157,7 à 172,9 μ) qui correspond vraiment aux ovocytes mûrs (fig. 3). Cette dernière population est très variable d'un animal à l'autre, étant fonction du temps écoulé depuis la dernière ponte.

A partir du mois de Septembre et en Octobre et Novembre on assiste à la disparition des ovocytes moyens, puis à la raréfaction des gros ovocytes et on retrouve peu à peu une prédominance d'animaux d'hiver: type que nous avons déjà défini.

DISCUSSION

Les faits principaux que nous pouvons dégager de ces observations en tenant compte aussi des résultats cytologiques obtenus par ailleurs sont les suivants:

En hiver, on ne trouve que des petits ovocytes de diamètre inférieur à 61 μ.

Au printemps les ovocytes sont de taille petite et moyenne (diamètre supérieur à 61 μ).

En été on rencontre toutes les tailles.

A l'automne on voit disparaître les ovocytes moyens et gros pour retrouver le type d'hiver.

Les fréquences maxima et minima ne sont pas distribuées au hasard; elles sont toujours localisées en des points ou des régions définis de l'échelle des diamètres.

Nous savons, par ailleurs, que l'ovaire a un fonctionnement continu et libère toute l'année des ovocytes dans le coelome.

L'analyse biométrique des cellules reproductrices coelomiques devrait donc, en toutes saisons, donner des résultats identiques. L'absence d'ovocytes de taille moyenne en hiver pourrait amener à penser que les petits ovocytes sont résorbés à cette période. Mais nous n'avons observé aucun indice de phagocytose ou de dégénérescence de ces cellules alors que le phénomène s'observe nettement pour les gros ovocytes rencontrés à l'automne ou en hiver et qui sont un reliquat de la période de reproduction précédente.

D'autre part il faut préciser que ces résultats n'ont qu'une valeur statistique, les diagrammes définis comme typiques de l'hiver ou du printemps ne se rencontrent que dans 90 % des cas environ au cours de ces saisons. Ces résultats avaient été obtenus en 1952; depuis lors nous avons étudié de très nombreux animaux, à d'autres fins, et nous avons constaté que d'une année sur l'autre le passage d'un type au suivant peut s'effectuer avec des décalages d'un ou deux mois. Cependant, animaux d'hiver, de printemps, d'été et d'automne, dans la mesure où l'on ne verra dans ces désignations saisonnières qu'une approximation, représentent des types très bien définis que nous avons retrouvés plusieurs années de suite avec les mêmes caractères. Ces observations n'ont été effectuées jusqu'à présent qu'à Roscoff et ne sont peut-être valables pour Phascolosoma vulgare que dans les conditions climatiques de cette région.

En tenant compte de ces différents éléments nous avons été amenés à assimiler la fréquence à l'inverse de la vitesse de croissance et à considérer ainsi qu'aux fréquences basses correspond une croissance rapide et aux fréquences élevées une croissance lente. D'une façon générale nous avons observé la présence de modes entre 34,2 μ et 45,6 μ et entre 153,9 et 165,3 μ, et des fréquences basses entre 90 μ et 140 μ; résultats qui sont transcrits en termes de vitesse de croissance dans le diagramme 4 (trait continu).

Les diagrammes successifs obtenus sur un même animal en hiver ne montrent aucune évolution significative, la croissance est donc lente en cette saison. L'absence de cellules d'un diamètre supérieur à 60 μ indique d'autre part l'existence, à cette taille, d'un blocage de la croissance. Ce fait doit être rapp-

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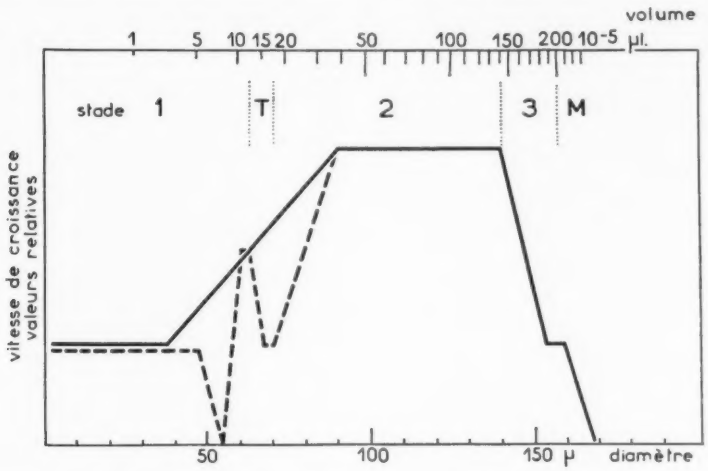

Fig. 4. Evolution de la vitesse de croissance en fonction de la taille des ovocytes ; *en tirets*, pour un ovocyte détaché de l'ovaire à l'automne ; *en trait continu*, pour un ovocyte détaché de l'ovaire en été.

roché des observations cytologiques qui montrent entre 60 et 70 μ (stade T) une évolution brusque et très particulière préludant à l'apparition des réserves lipidiques. Le stade T apparaît comme une phase critique sensible aux conditions extérieures.

La reprise de la croissance au printemps se traduit par l'apparition d'un deuxième mode à 66 μ. Dans l'hypothèse proposée nous aurions donc, après une période à vitesse de croissance faible jusqu'à 46 μ une brusque accélération (présence d'un minimum à 57 μ), ralentissement pour 66 μ puis enfin une nouvelle accélération de la croissance dont la vitesse reste élevée jusqu'à 140—145 μ (fig. 2 et fig. 4 tirets). Ce type de diagramme bimodal n'ayant été trouvé qu'au printemps, il semble logique de lui attribuer un rapport avec le phénomène de blocage qui a lieu pendant les mois précédents. Anticipant sur une publication ultérieure nous préciserons que les petits ovocytes d'hiver ont un caractère physiologique anormal; leur respiration endogène est plus élevée que celle des ovocytes de même taille étudiés au printemps ou en été. En Février et Mars chez des animaux passant du type hivernal au type de printemps on trouve une corrélation nette entre l'abaissement de la respiration endogène des cellules de moins de 60 μ et l'apparition de quelques stades T ou début de stade 2. Les mesures respiratoires étant faites sur plusieurs centaines de cellules il est impossible de préciser si les cellules, individuellement, présentaient des valeurs de respiration endogène intermédiaires ou si l'abaissement de cette respiration est brusque et complet. On pourrait admettre que la reprise de la croissance se fait en deux temps: transformation physiologique d'abord ame-

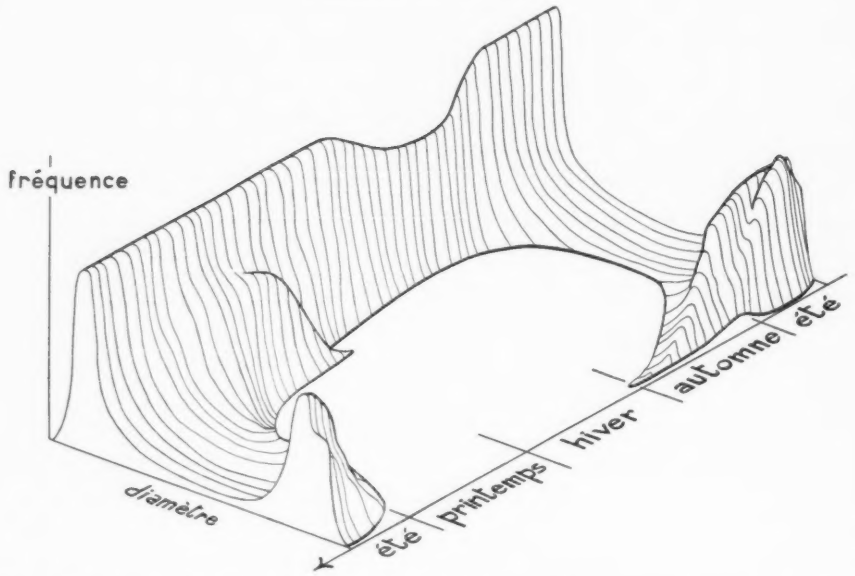

Fig. 5. Diagramme à 3 dimensions, groupant les courbes de fréquence successives obtenues au cours d'une année. La valeur absolue des fréquences est arbitraire ; les cloches correspondant aux petits ovocytes sont ramenées à une même hauteur sauf à l'automne où une décroissance rappele que l'ovaire est moins actif à cette époque.

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nant avec la disparition d'un caractère anormal la suppression du blocage hivernal et le passage du stade 1 au stade T ; puis après un temps d'arrêt (66 μ), passage du stade T au stade 2 (fig. 4 tirets). L'inflexion observable en toutes saisons dans la région qui va de 50 à 75 μ et qui traduit peut-être une pause dans l'accélération de la croissance au stade T, est alors renforcée par le passage d'une population homogène d'ovocytes et un mode apparaît à ce niveau.

Le mode correspondant au stade T ne s'observe plus en été, saison où très probablement la croissance est plus rapide et s'effectue sans temps d'arrêt à la fin du stade 1. Le passage d'un stade cytologique au suivant s'accompagne néanmoins toujours d'un changement dans la fréquence, c'est-à-dire selon notre hypothèse dans la vitesse de croissance. Au passage du stade 1 au stade 2 la croissance s'accélère (en hiver il y a blocage) ; elle se ralentit enfin, non pas pour des ovocytes mûrs, mais antérieurement lors du passage du stade 2 au stade 3 (fig. 3 et 4).

Les résultats que nous avons obtenus ici semblent donc confirmer la validité des stades que nous avons définis cytologiquement et leur donner une valeur plus générale, non seulement morphologique mais dynamique permettant de prévoir les correspondances physiologiques qui seront l'objet d'une publication ultérieure.

RESUME

On a étudié au cours de l'année les courbes de fréquence des tailles des ovocytes libres dans le liquide coelomique de Phascolosoma vulgare.

La période de reproduction s'étend de Juin à Septembre. L'ovaire a cependant un fonctionnement continu et libère toute l'année des petits ovocytes dans le coelome. On a reconnu que la croissance de ces ovocytes est, en hiver, bloquée à la fin du stade 1. Au printemps la reprise de la croissance et le passage au stade T se traduit sur les histogrammes par l'apparition d'un mode transitoire. Le stade T semble être une étape critique de la croissance.

La croissance en été s'effectue sans temps d'arrêt mais à des vitesses variables selon le stade. Le passage d'un stade au suivant est marqué par un changement de la vitesse de croissance: entre le stade 1 et le stade 2, accélération; vitesse élevée au stade 2; ralentissement au passage du stade 2 au stade 3.

Les différentes phases définies biométriquement sont en concordance avec les stades que révèlent les recherches cytologiques et physiologiques.

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THE DEVELOPMENT OF REISSNER'S FIBRE IN THE BRAIN OF THE SALMON

BY

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In a recent study of Reissner's fibre in the caudal part of certain lower vertebrates (Olsson 1955), I pointed out some facts concerning the development of the fibre in the brain. Thus the subcommissural cells, which are believed to be the producers of the fibre, could be identified already in a 3 mm *Xenopus* larva. However, it was quite impossible to trace any secretory substance in these cells until at the 6 mm stage, in spite of the fact that a fibre could clearly be seen in the caudal end of larvae only 2 mm in length.

Stages were also found with a fibre in the central canal and secretion-producing subcommissural cells, but with no fibre in the brain ventricle close to the subcommissural organ. I interpreted these facts as indicating that in the early stages the fibre-forming secretion is produced in regions other than the subcommissural organ. The purpose of the present investigation is to examine in detail the development of the fibre in the young brain.

MATERIAL AND METHODS

Salmo salar, 64 series between the neurula stage and fishes 75 mm in standard length.

Esox lucius, 6 specimens, the youngest just hatched, the oldest 42 hours older.

Petromyzon fluviatilis and *Xenopus laevis,* the young ammocoetes and tadpoles used for the paper Olsson 1955.

The material was fixed in Bouin's acetic acid—formol—picric acid fluid and

the fixing fluid according to BOUIN-ALLEN. Length means total length in cases where total and standard length are not both given. The specimens were measured in the antemedium (methyl benzoate—celloidin). The paraffine-embedded animals were cut in sections 4 μ thick, the adult brains somewhat thicker. The main staining technique was the chrome alum haematoxylin-phloxine method according to GOMORI (1941). For finer cell structures some sections were treated with the safraine waterblue—orcein—eosin method according to UNNA. Also used was the periodic acid—Schiff method (as "Dipas") according to HOTCHKISS (in EVERSON PEARSE 1954). In most cases these preparations were compared with unoxidized control sections of the same material. For purposes of comparison a large number of routine sections stained with iron haematoxylin and azan, both according to HEIDENHAIN, were used. Methods with no references above are described in ROMEIS (1948).

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DEVELOPMENT OF REISSNER'S FIBRE IN THE SALMON

In the 9 day (3.6 mm) stage, the anterior part of the nervous system is still a solid neural cord. In the region of the future deuterencephalon, this cord is medially divided by a cleavage, but the only existing ventricle is a small widening of this cleavage just above the floor cells. A diffuse secretory activity may be seen in some of the cells lining this ventricle, but a Reissner's fibre cannot be found at this stage.

The 11 day (5.0 mm) stage has already well-developed brain cavities and the brain has now reached the classic two-vesicle stage. A subcommissural organ cannot be found in the *archencephalon*. But nevertheless the central canal of the neural tube contains a well-developed Reissner's fibre. Frontally this fibre loses its thread-like form and becomes rough and uneven. It is possible to trace the fibre rostrally in *deuterencephalon* to the region of *plica ventralis encephalis*, where it disappears in a small accumulation of droplets with the same staining reactions as the fibre.

The 14 day (5.6 mm) stage has a well-developed subcommissural organ. Its cells have the typical, elongated nuclei and are situated rather far from the ventricular pole of the cells. Evidently, the subcommissural organ is a very active gland already at this stage. Its cells contain small chrome haematoxylin-positive granules, which are especially crowded as a ventricular border, and also a few phloxine-positive droplets. In the ventricle there is an accumulation of a substance consisting of small droplets. To judge by the position of this substance in relation to the subcommissural organ and its staining properties, there seems to be no doubt that it originates from this organ (cf. Fig. 4).

Reissner's fibre can be clearly seen in the central canal and the hind parts of the brain, but in the vicinity of the subcommissural organ no trace of it can

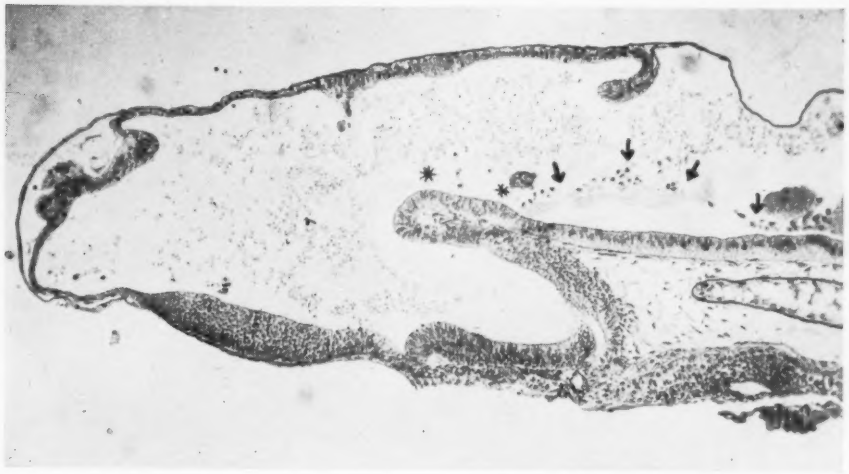

Fig. 1. *Salmo* 18 d. (7.4 mm). Median section; cf. Fig. 9 for references. Flexural organ between asterisks. Arrows indicate the row of "free cells" which shows the position of Reissner's fibre. Note the cell ball between asterisk and arrow. Cr.-htx., 4 μ 106 ×.

be seen. When followed from behind and cephalad the fibre is first found to be situated close above the floor cells in the central canal. In *rhombencephalon* it turns slightly dorsad, but in *mesencephalon* it turns ventrad again. It is now directed towards the ventral brain fold, *plica ventralis encephali,* divides and gives off several fine branches that spread over the cells of the ventral fold.

The course of Reissner's fibre at stages 15—21 days (7.0—8.5 mm). In all these specimens the fibre has almost exactly the same situation in the brain cavities (Fig. 1). When followed in longitudinal sections from behind and cephalad it is seen to turn dorsally, in the older specimens very steeply, towards the *plica rhombomesencephalica.* In one specimen (21 d.) two fibres make this dorsal curvature, but in the other the fibre is not divided until it has reached its dorsalmost point. From here it sends out a number of diverging branches in a brush-like manner. As in the 14 d. stage the branches are always directed ventrally and can be traced all the way to the cells of the ventral fold.

The "flexural" organ. It is evident that, at the above-mentioned stages, Reissner's fibre terminates at some cells of the ventral fold (here referred to as the "flexural" organ) instead of at the subcommissural organ, as at older stages. This flexural organ is situated in the floor of *mesencephalon* and consists of the tip of *plica ventralis,* i.e. about the basal plate cell region of the plica according to KINGSBURY (1922). In transverse sections it is seen to be restricted exclusively to the floor of the ventricle with no lateral extension (Fig. 2).

The cells of the flexural organ closely resemble those of the subcommissural

Fig. 2. *Salmo* 23 d. (8.4 mm). Transverse section through the flexural organ. Median cells with granulated ventricular border and fine fibrillae which soon gather to form Reissner's fibre (above in the ventricle). Cr.-htx., 4 μ. Gevapan, orange filter. 1,125 ×.

Fig. 3. *Salmo* 21 d. (8.5 mm). Median section through the flexural organ. The granules accumulate at the point where the fibrillae arise. Cr.-htx., 4 μ. Kodak P. 300, orange filter. 1,430 ×.

organ (Fig. 3). They are elongated ependymal cells, some of which at least reach the external limiting membrane with their basal feet. The nuclei are elongated and situated at some distance from the ventricle. The chrome haematoxylin-positive material is always situated in the central processes in the form of rather large granules. Close to the cuticular border the granules are tiny and densely packed. At the point where the fine fibrillae of Reissner's fibre arise

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the material forms small accumulations. Apparently the granules are as periodic acid—Schiff-positive as those of the subcommissural and infundibular cells. Exceptionally some secretory matter may be seen even in the peripheral processes, especially in the waterblue—orcein—eosin preparations. It is likely that some of the cells of the organ carry tall cilia, but it was not possible to determine whether the same cells are both ciliate and secretory.

It is often difficult to delimit the flexural organ caudally because the floor plate cells may show secretory characteristics far backwards. However, only the frontalmost part of the fold has any connection with Reissner's fibre and only this region, which usually forms a small eminence (Fig. 1), has been referred to here as the flexural organ. The floor cells behind the organ often contain blue granules (chrome haematoxylin) and have a deep blue ventricular border as far backwards as into the spinal cord.

The course of Reissner's fibre from the 22 d. stage (8.5 mm) onwards. Still at the 22 d. stage minute threads from the flexural cells unite to form a thin fibre. But this fibre soon unites with a slightly thicker fibre and then forms the normal Reissner's fibre. This thicker fibre can be traced frontad for a distance in the third ventricle but becomes invisible close to the subcommissural organ. It gives the strong impression of being a direct continuation of the secretion produced by the subcommissural organ.

The 22 ½ d. stage is very like the preceding stage, but the branch from the flexural organ is still thinner. At the 23 d. stage the flexural organ does not produce a fibre. The subcommissural organ produces thread-like secretion as well as droplets. The threads form the fine brush that converges to Reissner's fibre and the droplets often lie in rows on the threads.

At the 25 d. (10.0 mm) stage the brush of threads converges to two fibres that unite caudally (cf. *Ammocoetes*: NICHOLLS 1912). At the 64 d. (16 [18] mm) stage there are even three fibres frontally that have been reduced to two above the optic chiasma.

The subcommissural organ. The cells of the subcommissural organ undergo some interesting changes in structure during the development. Apparently they act as glandular cells very early and from the 14 d. (5.6 mm) stage on granules may be seen in their ventricular pole. The cells also contain a blue-stained (chrome haematoxylin) material, non-granulated in the light microscope (Fig. 6:1). Together with granules this substance is especially accumulated as a border lining the ventricle. There can hardly be any doubt that the subcommissural cells release a substance into the ventricle, where it forms conglomerates of droplets. These droplets have the same staining properties as the above-mentioned cell material and they are always found close to the subcommissural cells (Fig. 4).

At the 22 d. (8.5 mm) stage the subcommissural cells are no longer so

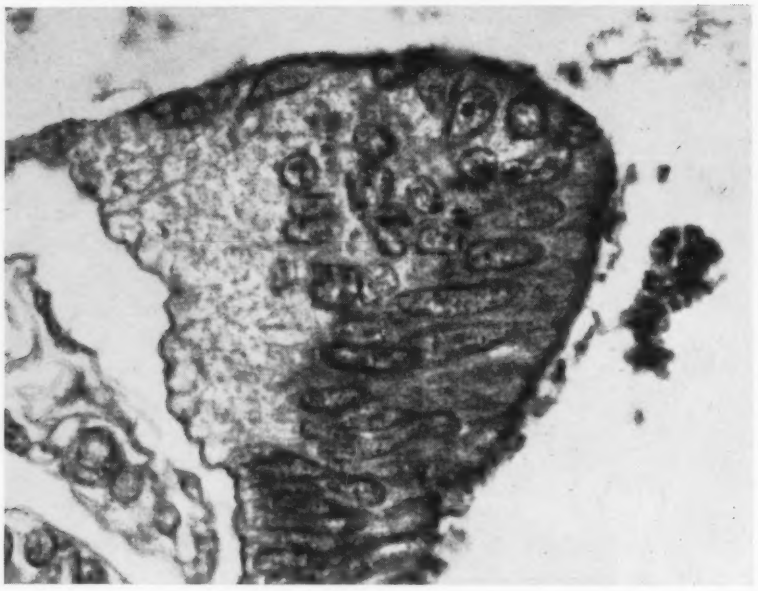

Fig. 4. *Salmo* 19 d. (7.7 mm). Sagittal section through a young subcommissural organ. Dark-staining central cell processes and secretory matter in the ventricle. Cr.-htx., 4 μ. Gevapan, orange filter. 1,232 ×.

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uniform as before. Compared with the younger stages two important differences are now seen. The granules in the ventricular pole have to a great extent been replaced by an extremely fine-granulated substance and now also the peripheral processes may contain granules, always in the form of rows. The Reissner's fibre fibrillae evidently arise from a certain type of cells (Fig. 6:3), while others (Fig. 6:2) still have the original mode of secretion in the form of droplets and granules into the ventricle. Evidently it is the first-mentioned cell type which contains peripheral granules. The cell region between the nucleus and the ventricular surface stains blue and the highest possible light microscope magnification shows that even this substance is composed of very fine granules. They concentrate as a ventricular border and form a darker staining accumulation at the point where the fine fibrilla of Reissner's fibre arises.

The second cell type (Fig. 6:2) has all stainable secretory matter centrally. The granules may be comparatively large and in essentials these cells are like the young subcommissural cells. At the 53 d. (12.0 mm) stage these droplet-forming cells can no longer be found in the subcommissural organ. At this stage, as well as at several other older stages, the basal granules are not situated in rows but are concentrated as a mass close to the basal pole of the nucleus (Figs. 5 and 6:4).

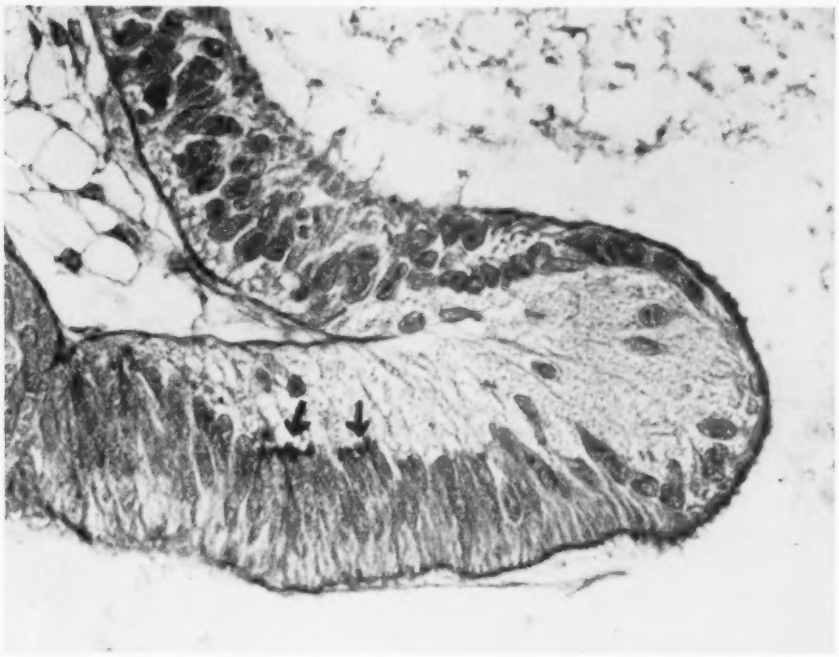

Fig. 5. *Salmo* 53 d. (12 mm). Sagittal section through a fibre-forming subcommissural organ. Peripheral secretion as granules close to the nuclei (arrows). Cr.-htx., 5 μ. Gevapan, dark orange filter. 675 ×.

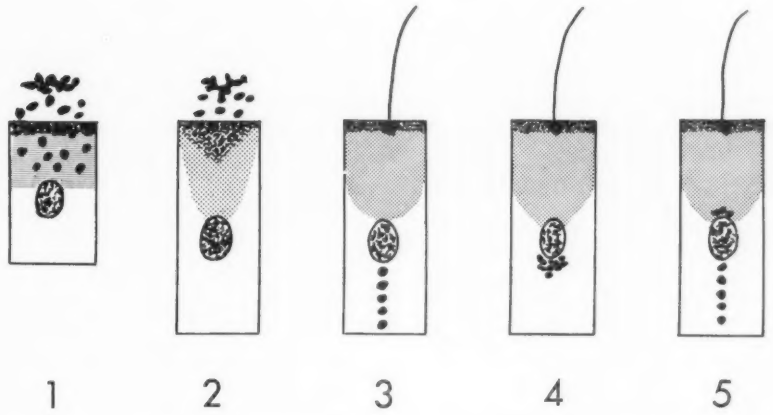

Fig. 6. Diagram showing subcommissural cells from *Salmo*. *1*. 14 d. (5.6 mm). *2*. and *3*. 22 d. (8.5 mm). *4*. 53 d. (12 mm). *5*. 108 d. (23/27/ mm). Not drawn to scale.

At still older stages large granules may also appear centrally to the nucleus. In these cases the granules are always situated close to the nucleus (Fig. 6:5).

The fate of the flexural organ at old stages. From the 23 d. stage on Reissner's fibre has no visible connection with the flexural organ. Nevertheless the cells still scantily contain chrome haematoxylin-positive granules. At the 53 d. (12.0 mm) stage there are no longer any granules but still a blue border in the floor cells far backwards in the brain. The 108 d. (23 [27] mm) stage lacks granules as well as the ventricular border in the floor cells, but the nuclei are still situated in the typical, basal position.

The "free cells". Different kinds of cells appear freely in the brain ventricles during the development. Young stages always contain several cell fragments, obviously originating from the eye stalks. Exceptionally also wandering blood corpuscles may be found here. Of particular interest in this connection are however the so-called "free cells" of AGDUHR (1922).

Already at the 11 d. (5.0 mm) stage a few free cells can be found close to Reissner's fibre. They are still rather scarce at the 13 d. (5.8) mm) stage, but the brain cavity now contains several balls of cells, which suggest a dense tissue more than a conglomerate of cells (Fig. 1). In one case such a cell ball was seen in contact with the ependyma as a tumour. Evidently the cell balls may release free cells into the ventricle.

At somewhat older stages the free cells increase in number and at the 18 d. (7.4 mm) stage a large number of cells are found close to the fibre following its course all over the ventricles (Fig. 1). Apart from a small accumulation of free cells near *lamina terminalis* the cells are found nowhere else in the ventricle except close to Reissner's fibre. The number of free cells decreases rapidly during the following days, but reaches a new peak at the 22 d. (8.5 mm) stage. The majority of the cells are now found along the new course of Reissner's fibre, i.e. between the flexural and subcommissural organs. At still older stages the cells rapidly decrease in number and a few days later they cannot be found any more. No doubt these cells play some role in the formation of the fibre, but it has not been possible to find out in what way.

THE FLEXURAL ORGAN IN OTHER ANIMALS

Petromyzon fluviatilis. Though a very careful re-examination was made of the ammocoetes material used for an earlier investigation (OLSSON 1955), it was not possible definitely to demonstrate the presence of a flexural organ in this animal. However, some floor cells in the brain of young animals seem to be secretory. Their nuclei are basally situated, the plasm often stains blue and they seem to discharge blue-stained (chrome haematoxylin) material into the ventricle. At the stages 214—307 h the fibre can frontally be traced close

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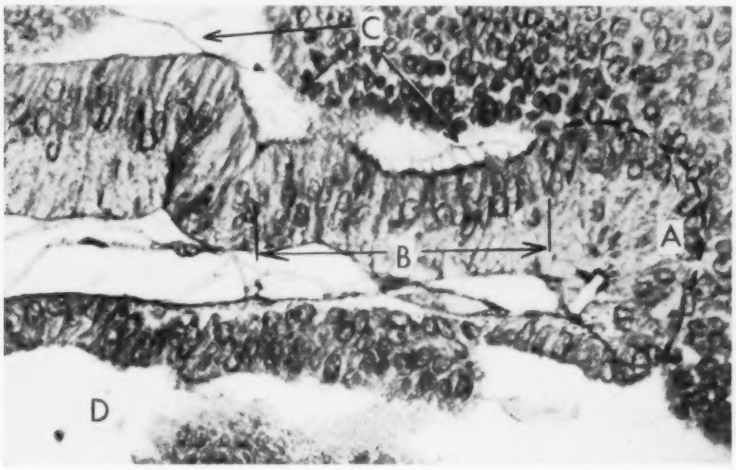

Fig. 7. *Esox*, newly hatched. Sagittal section through the ventral fold, showing A. Tip of the ventral fold. B. Flexural cells. C. Reissner's fibre. D. Infundibulum. Cr.-htx., 4 μ. Gevapan, dark orange filter. 600 ×.

to the floor cells but it suddenly disappears in the region of the above-mentioned cells, the situation of which may correspond to the flexural organ of the salmon. From stage 320 h on the fibre terminates at the subcommissural organ.

Esox lucius. The newly hatched pike has an easily recognizable flexural organ (Fig. 7). This does not, as in the salmon, form a small platform, but it has a typical wavy appearance in sagittal sections. The cells of the organ are also somewhat shorter than the tall median floor cells caudally. Ventricularly they contain chrome haematoxylin-positive granules and have a large accumulation of blue-stained material at the cell surfaces. From here fine threads are given off into the ventricle. Caudally the threads gather forming Reissner's fibre. In all the specimens the subcommissural organ is active, producing material in the form of fine droplets into the ventricle. In one specimen, a fine Reissner's fibre arises also from the subcommissural organ and this fibre together with that from the flexural organ forms the true fibre, i.e. the same condition as in the 22 d. old salmon.

Xenopus laevis. Already at the 2.5 mm stage some cells of the ventral fold give off a chrome haematoxylin-positive substance into the ventricle. At the 3.0 mm stage these cells have the typical basally situated nuclei. Their ventricular border now contains positive granules and threads are given off from the cell surfaces in the same manner as by the young pike. Reissner's fibre terminates close to these threads, but a direct continuation cannot be seen. Rostrally the ventral fold cells are sharply delimited but caudally the floor cells seem to have glandular properties far caudad. The subcommissural cells

contain positive granules at the 4.0 mm stage and at the 5.0 mm stage the fibre terminates here.

DISCUSSION

Flexural and subcommisural cells. It seems as though STUDNIČKA (1900) was the first observer to picture the special ependyma which DENDY & NICHOLLS (1910) named the subcommissural organ. In his description of the special features of the subcommissural epithelium STUDNIČKA remarks: "die ganz derjenigen ähnlich ist, welcher wir in den medianen Partien des Rückenmarkes begegnet haben, —." According to this author, these last-mentioned ventromedian cells are found also in the brain, but never so far ahead as into diencephalon. No doubt these cells, which he compared to the subcommissural cells, are identical with the cells of the flexural organ and its caudal continuation.

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After the present results it is clear that the flexural and subcommissural cells have more in common than merely a similarity in their cytology. In young animals Reissner's fibre terminates at the flexural organ, but in old animals it terminates at the subcommissural organ. The change of termination occurs rather suddenly, in *Salmo* at about the 22nd day (8.5 mm), in *Esox* soon after hatching time.

The flexural cells are ontogenetically the older of the two types. In *Salmo* at 11 d. they already produce droplets into the ventricle. At 13 d. to 23 d. Reissner's fibre arises from them and later on they do not show any ventricular secretion morphologically although they may contain secretory matter. The subcommissural cells are first distinguished at 14 d. and they produce droplets into the ventricle to 22 d. From now on Reissner's fibre arises from them.

Cytogenesis of the subcommissural cells. It is tempting to assume that the two types of subcommissural cells found at for instance the 22 d. stage only illustrate two different ways of releasing the secretion. In the young cells (Fig. 6:1—2) it may be given off from the whole cell surface. In the "adult" cells (Fig. 6:3—5) the releasing surface seems to have been restricted to only a tiny area of the cuticular border. The secretion is here given off as an endless fibre. The secretory matter in the central processes of these cells is extremely fine-granulated and concentrates close to the ventricular border, with an especial accumulation at the point where the fibre is given off. If large granules occur in the central processes, they are always found close to the nucleus and give the impression of being precursors of the fine-granulated matter, or perhaps, a kind of secretion store in cases of an over-production in relation to the restricted secretory release at the surface.

No cells in old *Salmo* stages seem to have preserved the juvenile form of

secretion as droplets and granules into the ventricle. The secretory cells with these properties described by BARGMANN & SCHIEBLER (1952), may however indicate the occurrence of such cells in mammals. If this is the case, it will perhaps explain the findings of BARGMANN & SCHIEBLER to the effect that Reissner's fibre does not arise from the whole surface of the subcommissural organ.

The question of a peripheral transport of secretory matter from the subcommissural cells has been investigated by OKSCHE (1954, 1956). In the *Salmo* material used by me the cells seem to reach the external limiting membrane and not to reach blood vessels. A relationship between the pineal and subcommissural organs is impossible in these young stages, because the pineal organ is still only a juvenile pouch when the first peripheral granules appear in the subcommissural cells. It seems more likely to me that the peripheral as well as the central granules only form a kind of store for the secretion which will later on be given off into the ventricle as the fibre.

An investigation by ARVY, FONTAINE & GABE (1955) demonstrates interesting variations in the secretory content of the subcommissural cells during the life-history of the larval and adult salmon. Also the illumination experiments on *Rana* made by LEGAIT (1942) and OKSCHE (1956) show the possibility of environmental influence on the subcommissural secretion. For the present egg stages of *Salmo*, however, the relation to the surroundings can be left out of consideration.

The relationship of the cells. The flexural cells of embryonic vertebrates, the infundibular cells of Amphioxus and the subcommissural cells of vertebrates all have positive chrome haematoxylin secretion. This does not mean of course that the cells produce identical secretions, but it seems unlikely that the composition of the different Reissner's fibres should vary within great limits. On the other hand the secretory material of these cells differs, as far as has been investigated, as regards alcohol solubility, PAS reaction after Bouin fixation (WISLOCKI & LEDUC 1954) and antidiuretic content (WINGSTRAND 1953) from the secretion of the neurosecretory praeoptic cells.

The vertebrate ontogeny shows a continuous reduction of chrome haematoxylin-positive ependymal cells. They are very common in the young medullary tube of *Xenopus* (OLSSON 1955). Later on the median floor cells and the flexural organ lose this kind of activity and finally the subcommissural organ is the sole remaining large area with these properties (cf. BARRY 1955).

It is possible that also the phylogeny has involved such a reduction of glandular elements in the brain (cf. EDINGER 1908 p. 163) and a specialization of the remaining gland cells. In fact there are some signs indicating that the organs here in question are descendants from larger glandular regions. The subcommissural organ has often a considerable lateral extension in the brain walls of lower vertebrates. The development and innervation of the infundibular

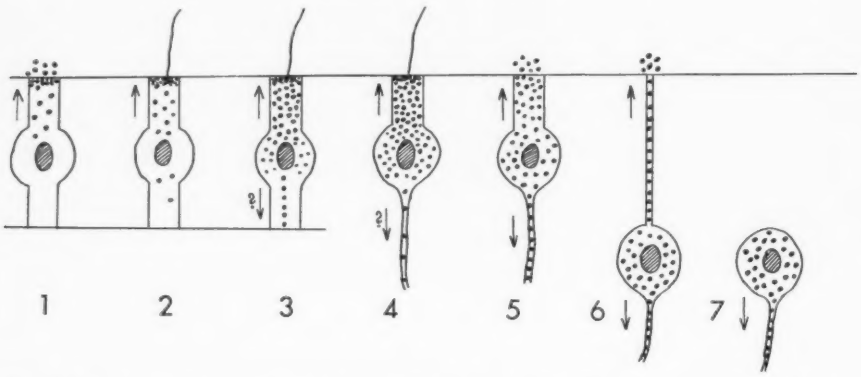

Fig. 8. Diagrams of some cells producing chrome haematoxylin-positive secretion. Arrows indicate direction of secretory release. *1.* Juvenile subcommissural or flexural cell. *2.* Flexural cell. *3.* Subcommissural cell. *4.* Infundibular cell (based on OLSSON & WINGSTRAND 1954). *5. 6.* and *7.* Neurosecretory praeoptic cells (based on OKADA et al. 1955). Not drawn to scale.

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organ points to a paired origin (BOEKE 1908). The flexural organ seems to be a strictly median structure, but in its direct continuation, latero-frontally in the brain walls, an (intercellular?) secretion is always found, the significance of which it has been impossible to interpret.

The juvenile flexural and subcommissural cells (Fig. 8:1) seem to be medullary tube glandular cells (MARCHETTI-XILO 1923) which are only slightly modified. Both develop in the same manner and finally release their secretion in the curious form of a fibre into the ventricle (Fig. 8:2—3). Eventually a peripheral release of secretory matter may even occur (OKSCHE 1954, 1956) (Fig. 8:3).

The infundibular cells (Fig. 8:4) are believed to be primary sense cells (BOEKE 1902, 1913, FRANZ 1923, 1927) but they also produce a Reissner's fibre (OLSSON & WINGSTRAND 1954). Neurosensory cells in the brain are usually derived from the ependyma (STUDNIČKA 1912) and I believe that we can look upon the infundibular cells also as descendants from the same glandular region as the flexural (and probably also the subcommissural) cells, but cells which have secondarily developed sensory characteristics without losing their glandular properties.

The neurosecretory praeoptic cells (Fig. 8:5—7) have been included in the series pictured here because OLSSON & WINGSTRAND derived them from infundibular cells. If their interpretation is correct, the glandular properties of the praeoptic cells are much older than the nervous properties, as has been pictured by SCHARRER & SCHARRER (1954) and discussed by CLARK (1956, two papers).

Even if no strict homologies may be demonstrated from this discussion it seems tempting to consider the infundibular organ in Amphioxus as the equi-

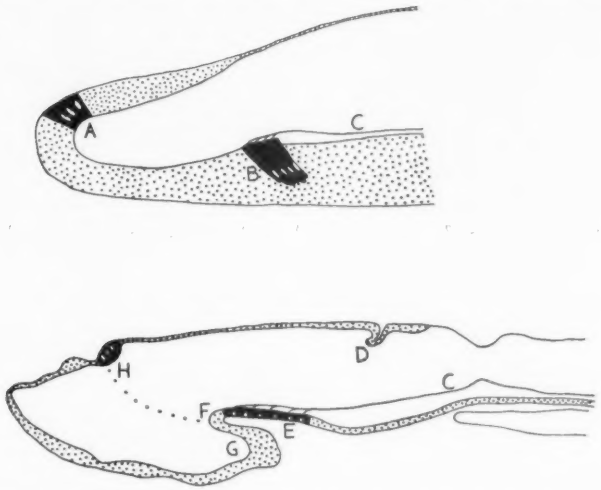

Fig. 9. Diagrams of the brain of Amphioxus (above) and the brain of a 14 d. (5.6 mm) salmon embryo (below). A. Secretory ependyma. B. Infundibular organ. C. Reissner's fibre. D. Plica rhombomesencephalica. E. Flexural organ. F. Plica ventralis. G. Infundibulum. H. Subcommissural organ. *Amphioxus* (based on OLSSON & WINGSTRAND 1954) 290 ×. *Salmo* 60 ×.

valent not only of the praeoptic neurosecretory cells and perhaps the saccus vasculosus cells (BOEKE; OLSSON & WINGSTRAND 1954 p. 10) but also of the flexural cells in the juvenile vertebrate brain and the subcommissural cells.

The brain of Amphioxus. The various attempts to homologize the brain (the word *brain* is here used simply in the sense of the terminal, modified part of the central nervous system; cf. v. UBISCH 1937) of Amphioxus with the brain, or part of the brain, of vertebrates have consisted in a search for corresponding regions in the two. OLSSON & WINGSTRAND found a Reissner's fibre in Amphioxus, but this fibre terminates ventrally at the infundibular organ (Fig. 9: B). The vertebrate fibre terminates dorsally at the subcommissural organ and the authors found it impossible to homologize two fibre-forming organs.

The annoying fact that Reissner's fibre arises dorsally in vertebrates but ventrally in Amphioxus (B) is less troublesome now that we know the flexural organ (E) in embryonic vertebrates. According to the discussion above we may consider the flexural and infundibular organs as partly corresponding regions. Nevertheless we are not much wiser concerning the homologies of the brains until we have discovered further corresponding regions.

The "cranial nerves" and the pigment spot of Amphioxus must still be considered very doubtful fixed points of this kind (see PIETSCHMANN 1929 and DRACH 1948 for references). The neuropores of vertebrates and Amphioxus are of course equivalents in the sense that they both indicate the point where

the medullary groove last closes terminally. But nothing indicates that this closure must occur in corresponding regions.

It is very tempting to compare the secretory ependyma in the neroporus region of Amphioxus (A) (Olsson & Wingstrand) with the young subcommissural organ (H). It must be pointed out that a homologization based only upon position and stainability with chrome haematoxylin may seem rather strained but the evidence for such a homologization is no weaker than that for most of the various other fixed points proposed. If the ependyma (A) is a subcommissural organ it seems probable that the brain ventricle of Amphioxus corresponds to the embryonic vertebrate deuterencephalon, as indicated by the dotted line between F and H.

It is interesting to note that in the close vicinity of the ependyma A in Amphioxus is situated the neuroporus, which is often considered to be an olfactory organ. Close in front of the subcommissural organ of vertebrates (H) develop the habenular nuclei' which are tertiary olfactory centres with a close connection to the flexural region (E) through the two *tr. habenulo-pedunculares*.

The embryonic Salmo brain. Let us sum up the considerations above with the aid of Figure 9. A slightly specialized glandular epithelium in the brain of a primitive form has been reduced and is found in vertebrates only as the subcommissural organ (H), but ontogenetically also as the flexural region (E). Some of the glandular cells have early acquired sensory characteristics and in Amphioxus this kind of cell is the sole (ventral) descendant, there forming the infundibular organ (B). In the vertebrate brain these sensory-secretory cells have specialized further and are found laterally as the neurosecretory praeoptic cells and, in some cases (according to Charlton 1932), ventrolaterally as the tuberis lateralis cells and ventrally as the saccus vasculosus cells (Boeke).

Many structures with secretory properties are found in the vicinity of the regions discussed here, as the *sulcus diencephalicus medius* (ref. in Ariëns Kappers et al. 1926, pp. 921, 971) and the various glandular roof organs both in front of the subcommissural organ and behind it (*Petromyzon*) (Tilney 1938). Several of them are perhaps the remains from a primitive glandular area, the position of which might have corresponded approximately to the region: the subcommissural organ—the two *tractus habenulo-pedunculares*—the flexural organ.

SUMMARY

Reissner's fibre was found in embryonic *Salmo* and *Esox* (and probably also in *Petromyzon* and *Xenopus*) to arise not at the subcommissural organ, as in old animals, but at a secretory ependyma in the floor of deuterencephalon, here referred to as the flexural organ.

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Also the subcommissural cells show secretory activity at a very early stage, but not until later do they seem to acquire a specialized method of secretory release in the form of Reissner's fibre. The flexural cells from now on gradually reduce their secretory activity.

The homologies between the infundibular organ in *Amphioxus* and the flexural organ in vertebrates, and a probable relationship between flexural, subcommissural, infundibular and neurosecretory praeoptic cells are discussed.

REFERENCES

Agduhr, E. 1922. Über ein zentrales Sinnesorgan (?) bei den Vertebraten. *Z. Anat. Entwgesch. 66.* Berlin.

Ariëns Kappers, C. U., Huber, C. G. & Crosby, E. C. 1936. The comparative anatomy of the nervous system of vertebrates, including man. *II.* New York.

Arvy, L., Fontaine, M. & Gabe, M. 1955. Modifications histologiques de l'organe sous-commissural au cours du cycle évolutif de Salmo salar L. *Arch. d'Anat. Micr. et de Morph. exp. 44.* Paris.

Bargmann, W. & Schiebler, Th. H. 1952. Histologische und cytochemische Untersuchungen am Subcommissuralorgan von Säugern. *Z. Zellforsch. 37.* Berlin.

Barry, J. 1955. L'hydrencéphalocrinie de substances neuro- et gliosécrétoire. *Biol. méd. 44.* Paris.

Boeke, J. 1902. Über das Homologon des Infundibularorganes bei Amphioxus lanceolatus. *Anat. Anz. 21.* Jena.

— 1908. Das Infundbularorgan im Gehirne des Amphioxus. *Ibid. 32.*

— 1913. Neue Beobachtungen über das Infundibularorgan im Gehirn des Amphioxus und das homologe Organ des Craniotengehirns. *Ibid. 44.*

Charlton, H. H. 1932. Comparative studies on the nucleus preopticus pars magnocellularis and the nucleus lateralis tuberis in fishes. *J. Comp. Neur. 54.* Philadelphia.

Clark, R. B. 1956. On the origin of neurosecretory cells. *Ann. des sc. nat., Zool. 11.* Paris.

— 1956. On the transformation of neurosecretory cells into ordinary nerve cells. *Kungl. Fysiografiska Sällskapets Förhandlingar. 26.* Lund.

Dendy, A. & Nicholis, G. E. 1910. On the occurrence of a mesocoelic recess in the human brain, and its relation to the sub-commissural organ of lower vertebrates; with special reference to the distribution of Reissner's fibre in the vertebrate series and its possible function. *Anat. Anz. 37.* Jena.

Drach, P. 1948. Embranchement des Céphalocordés. *Traité de Zool. 11.* Paris.

Edinger, L. 1908. Vorlesungen über den Bau der nervösen Zentralorgane des Menschen und der Tiere. *II.* Leipzig.

Everson Pearse, A. G. 1954. Histochemistry, theoretical and applied. London.

Franz, V. 1923. Haut, Sinnesorgane und Nervensystem der Akranier. *Jena Z. Naturw. 59.* Jena.

— 1927. Morphologie der Acranier. *Ergebn. Anat. und Entw.-gesch. 27.* München & Berlin.

Gomori, G. 1941. Observations with different stains on the human islets of Langerhans. *Amer. J. Path. 17.* Boston.

KINGSBURY, B. F. 1922. The fundamental plan of the vertebrate brain. *J. Comp. Neur. 34.* Philadelphia.

LEGAIT, E. 1942. Les formations épendymaires du troisième ventricule. *Thèse méd.* Nancy.

MARCHETTI-XILO, L. 1923. La secretione vesicolare durante lo sviluppo delle vescicole cerebrali nel Bufo vulgaris. Origine embrionale del liquido cephalorachidiano. *Arch. ital. di anat. e di embriol. 20.* Firenze.

NICHOLLS, G. E. 1912. The structure and development of Reissner's fiber and the subcommissural organ. *Quart. J. Micr. Sci. 58.* London.

OKADA, M., BAN, T. & KUROTSU. T. 1955. Relation of the neurosecretory system to the third ventricle and the anterior pituitary gland. *Med. J. Osaka Univ. 6.* Osaka.

OKSCHE, A. 1954. Über die Art und Bedeutung sekretorischer Zelltätigkeit in der Zirbel und im Subcommissuralorgan. *Anat. Anz. (Ergänzungsheft zum 101. Band).* Jena.

— 1956. Funktionelle histologische Untersuchungen über die Organe des Zwischenhirndaches der Chordaten. *Anat. Anz. 102.* Jena.

OLSSON, R. 1955. Structure and development of Reissner's fibre in the caudal end of Amphioxus and some lower vertebrates. *Acta Zool. 36.* Stockholm.

— & WINGSTRAND, K. G. 1954. Reissner's fibre and the infundibular organ in Amphioxus —results obtained with Gomori's chrome alum haematoxylin. *Univ. i Bergen Årbok (Publ. Biol. Stat.) 14.* Bergen.

PIETSCHMANN, V. 1929. Acrania. *Handb. d. Zool. (W. Kükenthal) 6: 1.* Leipzig.

ROMEIS, B. 1948. Mikroskopische Technik. München.

SCHARRER, E. & SCHARRER, B. 1954. Hormones produced by neurosecretory cells. *Recent Progr. Hormone Research. 10.* New York.

STUDNIČKA, F. K. 1900. Untersuchungen über den Bau des Ependyms der nervösen Centralorgane. *Anat. Hefte. 15.* Wiesbaden.

— 1912. Über die Entwicklung und die Bedeutung der Seitenaugen von Ammocoetes. *Anat. Anz. 41.* Jena.

TILNEY, F. 1938. The glands of the brain with especial reference to the pituitary gland. *Ass. for Res. in Neur. and Ment. Dis., Res. Publ. 17.* Baltimore.

UBISCH, L. VON, 1937. Ist das "Gehirn" von Branchistoma primitiv oder rudimentär? *Z. wiss. Zool. 150.* Leipzig.

WINGSTRAND, K. G. 1953. Neurosecretion and antidiuretic activity in chick embryos with remarks on the subcommissural organ. *Ark. f. Zoologi. 6.* Stockholm.

WISLOCKI, G. B. & LEDUC, E. H. 1954. The cytology of the subcommissural organ, Reissner's fiber, periventricular glial cells and posterior collicular recess of the rat's brain. *J. Comp. Neur. 101.* Philadelphia.

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ON THE PERIPHERAL AUTONOMIC NERVOUS SYSTEM OF THE TRUNK REGION OF LAMPETRA PLANERI

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I. INTRODUCTION

Information regarding the autonomic nervous system in lampreys is generally very imperfect (cf. NICOL 1952). Concerning the innervation of the alimentary canal the facts known are that in the adult fibres from the vagus nerve reach the anterior portion of the oesophagus (foregut) and that these fibres run on each side of the oesophagus to the limit between this portion of the alimentary canal and the dilated posterior portion, the intestine (LANGERHANS 1873, TRETJAKOFF 1927). Caudally to this limit a nervous plexus formed by multipolar ganglion cells has been observed in the wall of the alimentary canal (LANGERHANS 1873, KRAUSE 1923, TRETJAKOFF 1927, cf. also literature reviewed by NICOL 1952). There is, in the literature, much controversy regarding the innervation of the heart. CORDS (1929) completely denied the existence of vagal intestinal branches behind the branchial basket. A description of the central connections and of the peripheral ganglion cells of the heart has, however, been given recently (AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND, 1956). In the head region the structure of the sympathetic nervous system is fairly well known (JOHNSTON 1905, TRETJAKOFF 1927, LINDSTRÖM 1949). RANSON & THOMPSON (1886) expressed the opinion that a commissural sympathetic system is missing in the trunk region. JULIN (1887) described structures in the body of lampreys which he considered to be autonomic ganglia arranged segmentally but lacking longitudinal connections. GIACOMINI (1902) and GASKELL (1912) gave evidence which

strongly indicated that these structures belonged to the interrenal system. Information about details of the autonomic nervous system of cyclostomes has been given also by several other authors (literature in NICOL 1952) but in any case "regrettably little is known about the organization in this group" (NICOL 1952, p. 3).

II. MATERIAL AND METHODS

The material consists of 33 series of sections of larval and adult specimens of Lampetra planeri, fixed in Bouin's fluid, Bodian's fluid no. 2 or a sublimate-acetic acid fixative according to PALMGREN (1955). Pieces of the trunk measuring 1—2 cm from the posterior branchial and heart region, the middle part of the body, the cloacal region, and the tail were embedded in paraffin, often over methyl benzonate, and sectioned transversally, sagittally, or horizontally (8—15 microns). The preparations were stained according to BODIAN (1936, 1937), according to the modification of this method by ZIESMER (1951), or, mostly, according to a modification of the Bodian method which is particularly suitable for the study of peripheral nerves (JOHNELS 1955). Silver preparations according to PALMGREN (1948, 1955) and Bielschowsky (AGDUHR 1930) were also employed.

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III. THE VAGUS INNERVATION OF THE ALIMENTARY CANAL AND THE HEART

There are great differences in structure as well as in function of the alimentary canal of the larval and the adult lamprey (cf. BOENIG 1927, KEIBEL 1927, PIETSCHMANN 1933). Thus, the anterior portion of the oesophagus of the adult is formed during the metamorphosis in the dorsal wall of the branchial region, and is completely lacking in the larva; in the larva the endostyle apparatus functions as a part of the alimentary system, but changes into the much smaller thyroid gland of the adult during the metamorphosis (cf. KRAENTZEL 1933). The cavity of the intestine is simple in shape in the larva, forming a simple u around the spiral valve as seen in a transverse section (cf. Fig. 6). In the adult a large number of secondary folds in the mucosa of the spiral valve as well as of the wall of the gut make the shape of the cavity much more complicated (cf. Fig. 9). In the larva the spiral valve starts on the left side in the rostral part of the intestine, and in this place the entrance of the ductus choledochus is situated. In the adult the base of the spiral valve has turned to the right side at its rostral end, and the ductus choledochus has disappeared completely during the metamorphosis. At the same time the surface of intimate connection bet-

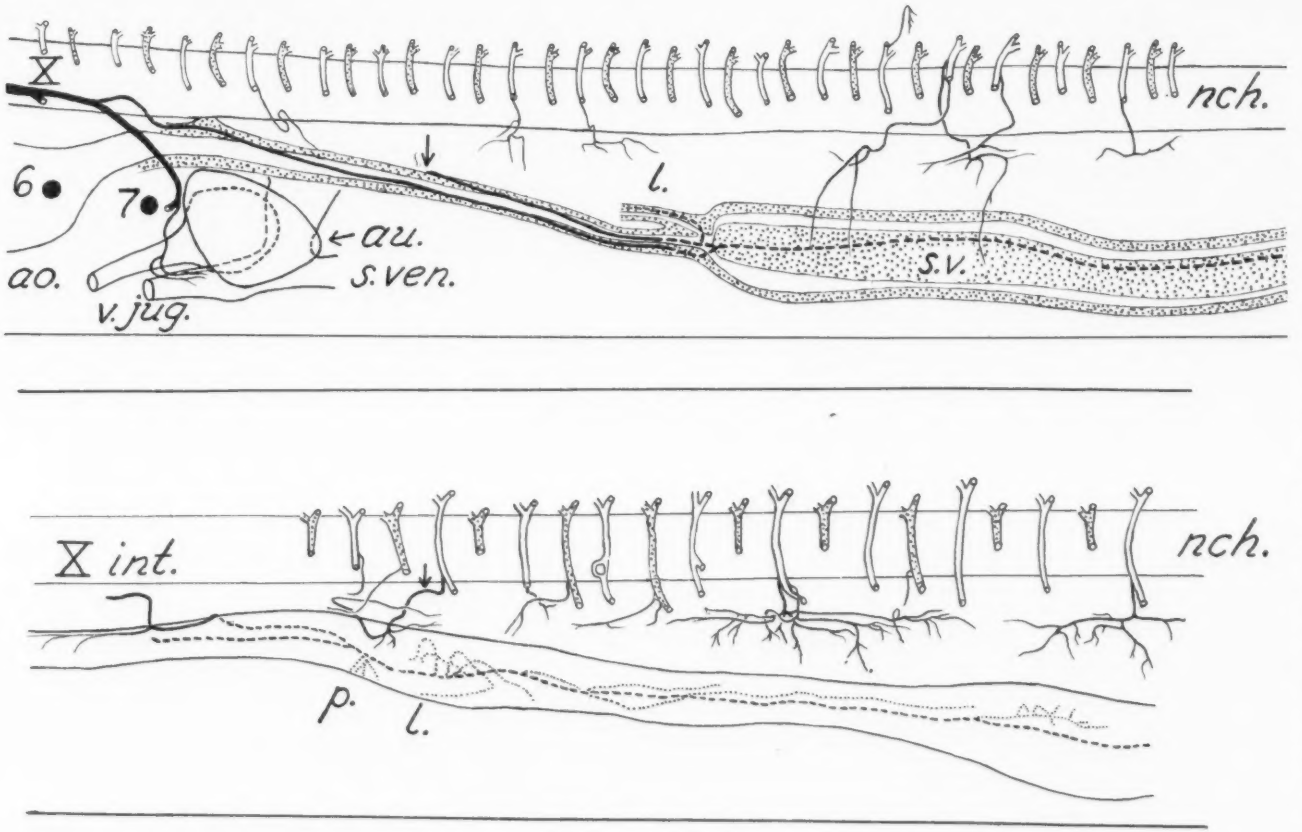

Fig. 1. The autonomic innervation in the postbranchial portion of the trunk of Lampetra planeri. Reconstructions. Lateral view of the left side. a. Larva, 10 ×. b. Adult, 15 ×. In a. the arrow indicates the right intestinal nerve turning to the left side. In b. the arrow indicates the position of the small nerve mentioned in Fig. 13. ao. aorta, au. auricle, nch notochord, l. liver region, p. pericardial region, s.v. spiral valve, s.ven. sinus venosus, v.jug. vena jugularis impar, X. nervus epibranchalis vagi, X int. intestinal branch of vagus, 6, 7, the sixth and seventh branchial openings. Ventral and pertaining rami dotted.

ween the oesophagus and the liver is enlarged. Dorsally, this area is connected with the subchordal structures by means of a short but broad mesenterium. Apart from this there are, in the adult as well as in the larva, only very small fragments of a dorsal and still smaller ones of a ventral mesenterium situated close to the anterior and posterior ends of the alimentary canal (cf. MARINELLI & STRENGER 1954). Thus, the alimentary canal is fixed to the rest of the body in the rostral and caudal ends only. In spite of all these differences the main features of the innervation of the alimentary canal are very similar in the larva and the adult.

As observed by LANGERHANS (1873), TRETJAKOFF (1927) and others the intestinal branch of the vagus nerve ramifies from the main vagus trunk above the sixth branchial arch (Fig. 1). From here it runs for a short distance caudally, then in a curve medially and enters the dorso-lateral part of the wall of the oesophagus. In the larva this entrance is close to the opening of the oesophagus in the posterior part of the branchial chamber. In the adult branches from the intestinal nerve are directed rostrally from this place into the wall of the suprabranchial part of the oesophagus which is formed during the metamorphosis. The main bundle of the vagus intestinalis is, however, directed caudally also in the adult.

In the larva the right intestinal nerve turns in a long spiral from the right side over the dorsal side to the left side (indicated by an arrow in Fig. 1 a). The left nerve runs close to the ventral surface of the oesophagus to the limit between this part of the alimentary canal and the intestine. Immediately in front of this limit the originally right branch sends a small branch towards the ligament which, in the larva, connects the liver with the intestine and contains the ductus choledochus, the arteria intestinalis, and the vena intestinalis. This liver branch was observed by LANGERHANS (1873). In the literature the opinion is expressed that the intestinal branches in this place dissolve into a diffuse nerve cell plexus typical of the intestinal wall (LANGERHANS 1873, TRETJAKOFF 1927, LINDSTRÖM 1949). However, it is easy to observe, in the present material, that the two intestinal nerves turn more deeply into the tissue of the wall and unite into a single nerve at the beginning of the spiral valve. From here this single nerve runs in the spiral valve very far towards the caudal part of the intestine (Fig. 2 a). Fibres are distributed to various parts of the spiral valve and through the base of the spiral valve towards the surface wall of the intestine. The tissue which surrounds the intestinal artery and vein in the spiral valve is haemopoietic (JORDAN & SPEIDEL 1930), and it is a well known fact that the observation of nerve fibres as well as of ganglion cells by means of silver methods is rendered rather difficult in tissues rich in blood or blood cells (cf. TRETJAKOFF 1927). This difficulty is eliminated in the present material by means of the aniline oil alcohol reduction (cf. JOHNELS 1955).

In the adult the intestinal branches from the main vagus trunk are thicker

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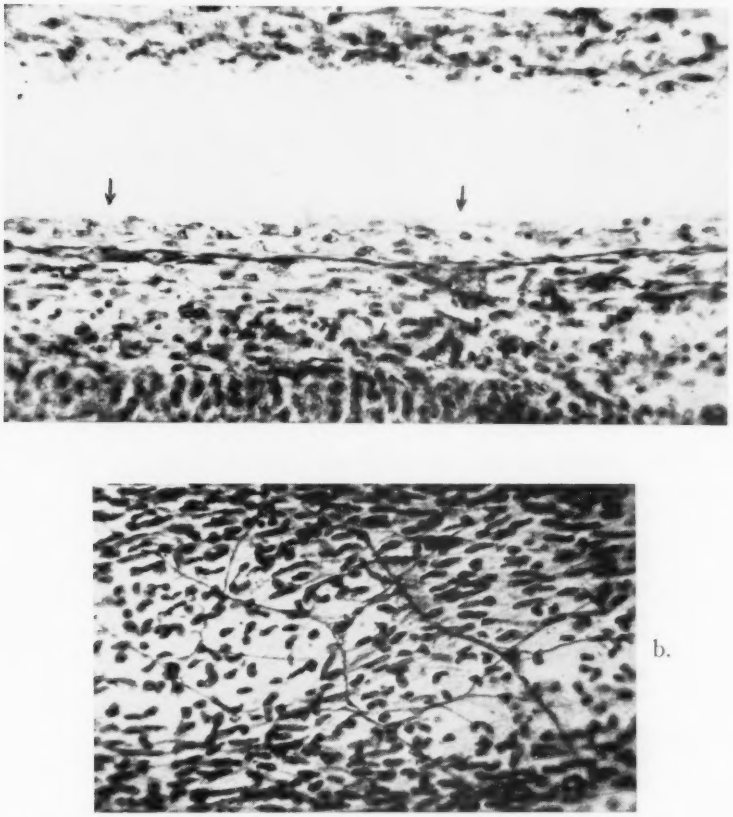

a.

b.

Fig. 2. Longitudinal sections through intestinal spiral valve. From the middle portion of the intestine of a larval Lampetra planeri. Microphotographs, 300 ×. a. Section through the vagus intestinalis impar. Two accumulations of ganglion cells (indicated by arrows) and a few nerve fibres are seen. The empty space is the lumen of the intestinal vein. b. The enteric nerve plexus.

and consist of a larger number of nerve fibres than in the larva. Also many of the fibres in the intestinal and heart branches are thicker in the adult than in the larva. In conformity with the position of the starting point of the spiral valve in the adult (see above) the left intestinal branch turns dorsally and over to the right side (Fig. 1 b). In this position the two branches run for a considerable distance separated from each other in the mesenterial connective tissue which keeps the oesophagus tube fixed to the liver surface. Several small nerves or isolated fibres are distributed to the liver. Many of the liver nerves run towards the arteria coeliaca and along this vessel into the liver tissue. In the adult a few small branches have been observed which seem to be directed towards the dorsal parietal wall of the pericardium (cf. Fig. 1 b).

The limit between the oesophagus and the intestine is obscure in the adult

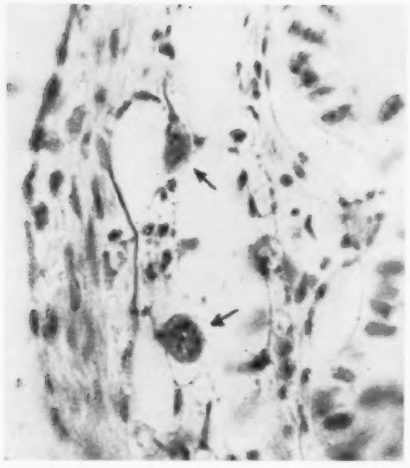

Fig. 3. Two ganglion cells from the rectal portion of the intestine. Muscular layer to the left, base of intestinal epithelium to the right. Microphotograph, 680 ×.

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owing to changes during the metamorphosis which have resulted in the transformation of the larval oesophagus into a structure which may be termed the foregut in the adult. However, also in the adult the two vagus branches unite into a single nerve between the foregut and the intestine. This nerve runs caudally in the tissue of the spiral valve in the adult as in the larva.

LANGERHANS (1873) and TRETJAKOFF (1927) mentioned the presence of ganglion cells in the intestinal branch of the vagus nerve. Ganglion cells in the wall of the intestine were observed also by KRAUSE (1923). In the present material there are small multipolar cells to be seen in all parts of the alimentary canal in the tissue of the spiral valve (Fig. 2 b) and in the surface wall of the intestine and the foregut. There are also somewhat larger ganglion cells which appear to be bipolar situated in the intestinal nerve branch (Fig. 2 a). Particularly large numbers of these ganglion cells are situated in the place where the liver nerves branch off and at the point of junction of the two intestinal branches. The intestinal type of ganglion cell is present also in the rectal part of the intestine (Fig. 3).

Comparatively large accumulations of cells which seem to be of the same type as the chromaffin cells described by GIACOMINI (1902) and GASKELL (1912) are situated within the liver close to the dorsal mesenterium, and smaller groups of these cells are present in the rostral part of the spiral valve of the intestine.

The nerve fibres to the heart run in the epibranchial trunk of the vagus nerve (Fig. 1 a). The heart branch separates from the vagus caudally to the hindmost branchial opening and runs ventrally to the wall of the median (ventral) jugular vein. The fibres form a network in the wall of the vein but run in a mainly caudal direction and reach the wall of the sinus venosus where

they spread. In the wall of the median jugular vein and in all parts of the heart there are small cells which become stained with silver. These cells seem to be identical with chromaffin cells which have been observed—as far as the heart is concerned—in the sinus venosus by several authors but which are present close to the interior surface also in the wall of the auricle and the ventricle. These cells form a continuum throughout the heart and are possibly of a neuronic nature. There is also a small number of larger ganglion cells in the heart. For further information see AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND (1956).

IV. THE AUTONOMIC SYSTEM IN CONNECTION WITH SPINAL NERVES

In the present investigation three different types of autonomic nerves or fibres emerging from spinal roots are considered.

(1) In accordance with the fact that there is no mesenterium along the intestine apart from small fragments close to the ends the nerves or nerve fibres which reach the alimentary canal enter at the anterior end, from the vagus, or at the posterior end of the gut (cf. p. 270 ff.). There are, thus, no visceral branches which arise from the spinal nerves and proceed to the digestive tract between these places as has been assumed in the literature (cf. LINDSTRÖM 1949, NICOL 1952). The autonomic fibres in the main part of the trunk are, thus, distributed to the kidneys and the gonad, and also to the walls of arteries in the body wall.

(2) The chromaffin elements the distribution of which has been described by GIACOMINI (1902) and GASKELL (1912) are at least mainly innervated by nerve fibres leaving the central nervous system along the dorsal roots.

(3) In the vicinity of the cloacal region generally, and particularly in the tissues around the urogenital sinus, the urogenital pore, and the excretory ducts, a very complicated system is formed by nerves and ganglion cells (DOHRN 1886, 1888).

The autonomic nature of these three groups of nerves is, with certain exceptions in the third case (cf. p. 276), easily determinable. Autonomic elements have been described also in other parts of the body of the lamprey (cf. FUSARI 1907 a, b, RAZZAUTI 1917), but these fibres are extremely difficult to distinguish from somatic fibres and they are not considered in this work.

1. THE INNERVATION OF THE GONAD, THE KIDNEYS, AND SEGMENTAL ARTERIES

The large vessels of the body, the dorsal aorta and the spacious cardinal veins, run longitudinally in the body ventrally to the notochord. These vessels

and the surrounding connective tissue are separated from the viscera situated ventrally in the body cavity by a horizontal membrane of dense fibrous connective tissue. This membrane in this paper is called the *horizontal membrane*. Laterally it is connected with the fibrous connective tissue membranes which, medially covered by the parietal peritoneum, line the lateral wall of the body towards the visceral cavity. These membranes are termed the *parietal membranes* (cf. Figs. 6, 7, and 9). Along the dorsal margin these parietal connective tissue membranes are on each side fastened to the ventrolateral surface of the notochord. These membranes, the horizontal and the two parietal ones, play an important role as passage-ways for sympathetic fibres (cf. pp. 259, 271). It seems to be a rule that nerve fibres or nerves which enter into and run within the tissue of these connective tissue membranes sooner or later turn into the viscera and ramify there. The difficulty of staining these nerve fibres selectively from the surrounding fibrous connective tissue seems to be the explanation why they have escaped observation by other authors on the neurology of lampreys. These visceral fibres are most easy to locate in the places where they deviate from the ventral ramus of the spinal nerves in order to enter the visceral tissues. This place is either close to the ventrolateral surface of the notochord where the parietal connective tissue membrane is fastened or at the place of union of the horizontal and the parietal connective tissue membranes (cf. Figs. 6 and 9).

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It has been stated earlier that autonomic fibres only arise from dorsal roots in lampreys (FÜRBRINGER 1897, GOODRICH 1930). However the present study has revealed the important fact that autonomic fibres emerge from ventral roots as well (see p. 262).

With few exceptions there are usually only one or two nerve fibres which deviate from the rest of a ventral spinal ramus and turn towards the viscera. There are, thus, no real visceral "nerves" in the trunk region of lampreys (cf. however IV, 3). It seems that most of the dorsal roots of the trunk region contain such autonomic fibres. In the tail they seem to be less common. Autonomic fibres in ventral roots have been observed only in adult specimens. They appear to be less frequent than those in dorsal roots. The innervation of the viscera is, thus, not strictly segmental but more "generalized" than the somatic innervation.

Apart from the fibres which are directed towards the cloacal region it is usually easy to see whether there are autonomic fibres present in dorsal roots. These autonomic fibres are mostly distinctly thicker than the rest of the dorsal root fibres (Fig. 4). However, some of these thick fibres may innervate chromaffin cells (see p. 266). The rest of them will rum to arterial walls or to the viscera. Usually there are only one or two such visceral fibres emerging from each root. Very often a thick fibre is accompanied on its course by an extremely thin fibre (Fig. 5) which can be traced running along the thick one into the

Fig. 4. Fibres of a dorsal root cut longitudinally in a transverse section of the body. The visceral fibre is considerably thicker than the other fibres. To the right of the fibre bundle a dense layer of chromaffin cells situated along a segmental vein (not seen in the section) in a surface section. To the right the elsatica of the notochord. Microphotograph, 340 ×.

visceral tissues. Unfortunately, owing to observation difficulties caused in the first place by the large number of pigment cells, it has not been possible to judge whether the thin fibres follow the ramifications of the thick ones as far as the terminations but often they have been traced together for considerable distances. However, thick as well as thin fibres have been observed isolated from each other in the horizontal membrane and other localities.

The course of sympathetic fibres to the viscera follows two different routes. A few leave the ventral ramus of the spinal nerve laterally to the notochord and run dorsally to the cardinal vein in the thin fibrous membrane between this vessel and the notochordal sheaths. They pass further between the cardinal vein and the aorta and reach the horizontal membrane close to the mid-line of the animal (Fig. 6). In this membrane branches run anteriorly and posteriorly, often for considerable distances (the horizontal part of the branches in Fig. 1)

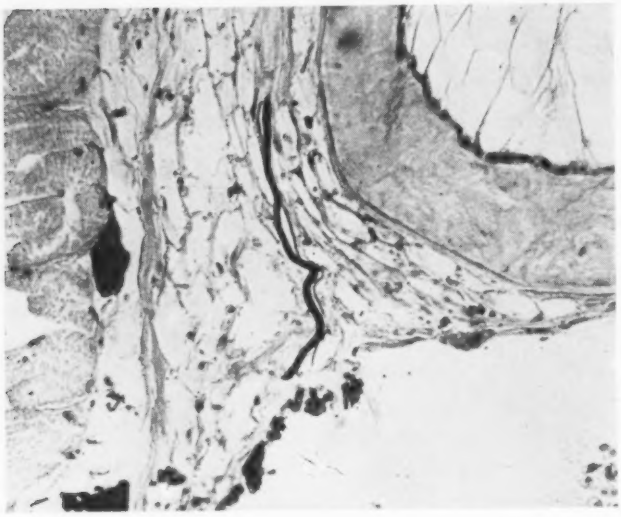

Fig. 5. Thick and thin visceral fibres. To the right above the notochord; below the cardinal vein. Microphotograph, 340 ×.

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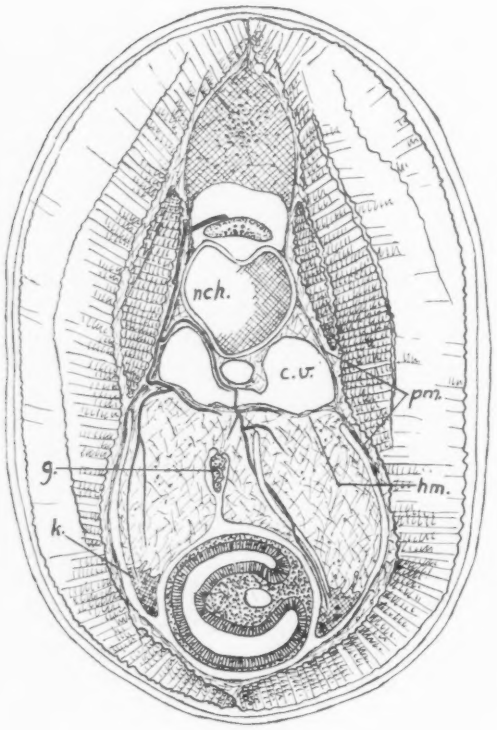

Fig. 6. Drawing illustrating the course of visceral fibres in the trunk region of a larva. Projection on a transverse section of the two fibres which in Fig. 1 a originate from the same dorsal root. 20 ×. c.v. cardinal vein, g. gonad, hm. horizontal membrane, k. kidney, nch. notochord, pm. parietal membrane.

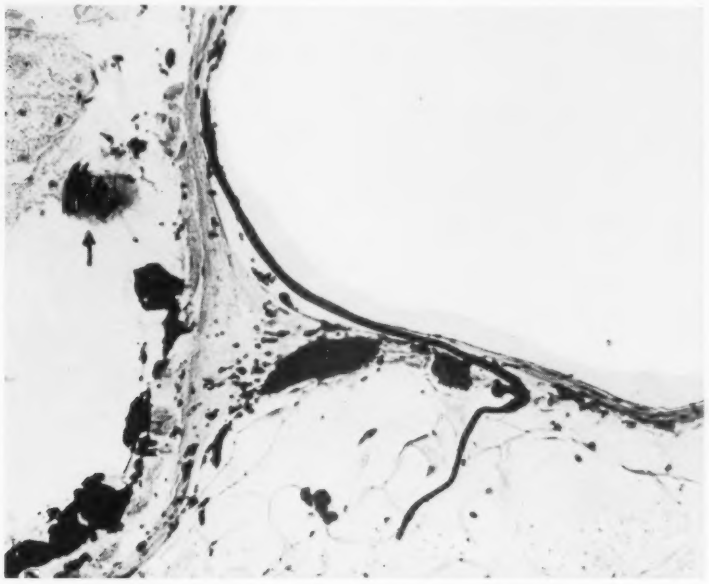

Fig. 7. Thick visceral fibre from dorsal root in the trunk region entering the adipose tissue ventrally to the horizontal membrane. From a transverse section. The cavity of the posterior cardinal vein in the upper right corner. The sectioned fibres of the dorsal root visible in the dark spot to the left (indicated by arrow). Microphotograph, 340 ×.

sending branches ventrally into the peculiar haemopoietic adipose tissue which fills the visceral cavity dorsally to the kidneys. Usually these fibres run along small arteries which are destined to the kidneys and some of these branches have been traced as far as into the kidney parenchyma. This type of fibre which enters the viscera dorsally to the cardinal vein as a rule terminates in the opposite side of the body and thus crosses the mid-line of the body.

Most sympathetic fibres from dorsal roots enter the visceral cavity ventrally to the cardinal vein (Figs. 6 and 7). These fibres also run into the horizontal connective tissue membrane entering its tissue at the place of junction with the parietal connective tissue membrane. In this case, too, the branches usually run for some distance caudad and craniad within the membrane before penetrating ventrally into the loose adipose tissue and the kidney parenchyma.

Several of the thick fibres of the dorsal roots do not enter the visceral cavity. Some of them are concerned with the innervation of chromaffin cells (see upp. 266 ff.). Other thick fibres branch within the dorsal or ventral ramus and in this way assume approximately the same thickness as the rest of the dorsal root fibres. Consequently it is often impossible to trace these fibres to their terminations in order to ascertain their nature. In especially favourable cases, however, it has been possible to follow these branches and in all these cases a delicate system of thin terminal branches has been observed on walls of

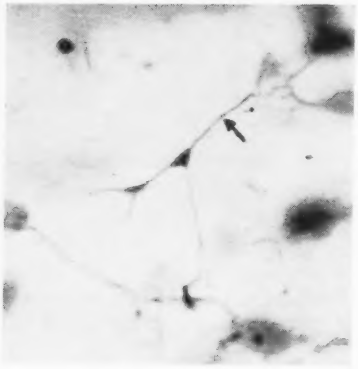

Fig. 8. Terminal branches in the wall of a segmental artery in the body wall. Microphotograph, 1 800 ×.

segmental arteries (Fig. 8). It seems that these fibres are arterial vasomotor. In a few cases thick fibres have been observed to give off such arterial branches before entering the horizontal membrane and the viscera. Probably many (all?) of the fibres which terminate exclusively in the viscera are also vasomotor.

From the dorsal ramus of spinal nerves thick fibres have been observed to enter the haemopoietic tissue dorsally to the spinal canal and to terminate in fine branches (one case is reconstructed in Fig. 1 a).

In some cases the fibres which originate in the ventral roots are of the same thickness as the thick ones of the dorsal roots. Mostly, however, they are considerably thinner but, on the other hand, always distinctly thicker than the thin visceral fibres accompanying the thick ones from the dorsal roots which were mentioned above.

In ventral roots and ventral spinal nerves it is impossible to distinguish between the sympathetic fibres and the rest of the fibres of the root. This is due to the fact that the ventral roots and pertaining rami always contain fibres or branches of fibres of a highly varying thickness. Further, the number of fibres in ventral root rami is much larger than in those of dorsal roots. The autonomic fibres of ventral roots can be most easily observed peripherally, especially where they enter the connective tissue membranes. From here the fibres can be traced to the ventral rami of the ventral spinal root where they join the fibre bundles. It has proved impossible, however, to state positively that the fibres follow the ventral rami all the way into the spinal cord owing to the large number of whorled motor fibres. On the other hand nothing has been observed to indicate that this might not be the case. Peripherally they are often difficult to follow for a more considerable distance. In Fig. 9 a case is pictured in which a fibre from a ventral root was followed up to the connective tissue septa of the gonad. This fibre is peculiar—as compared with the fibres emerging from dorsal roots—in terminating in the same side of the trunk as that from which it emerged from the central nervous system although it has

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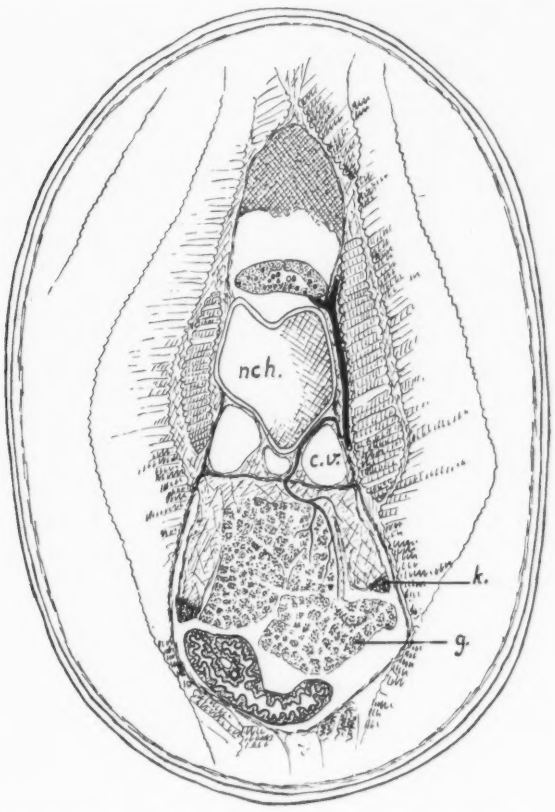

Fig. 9. Drawing of gonad branch from ventral root in adult specimen. Projection on a transverse section. 20 ×. c.v. cardinal vein, g. gonad, k. kidney, nch. notochord.

passed dorsally to the cardinal vein. The presence of autonomic fibres in ventral roots has been observed only in adult animals.

There are small cells in the gonad and the kidney parenchyma which may be peripheral sympathetic neurons. They are multipolar and form plexuses very similar to that of the intestine. In some cases slightly larger cells have been noticed which also may be peripheral sympathetic cells. In a few cases similar cells have been observed also within the connective tissue of the horizontal membrane.

The fibres which terminate in arterial walls have thin terminal branches which are characterized by the presence of small expansions, particularly where they branch (cf. Fig. 8). The same has been observed in the fat tissue dorsally to the kidney parenchyma (Fig. 10). No peripheral ganglion cells have been observed which might be suspected to influence arterial walls.

The fact that the autonomic nervous system is not as strictly segmented as the somatic system is indicated not only by the irregular outflow of the fibres

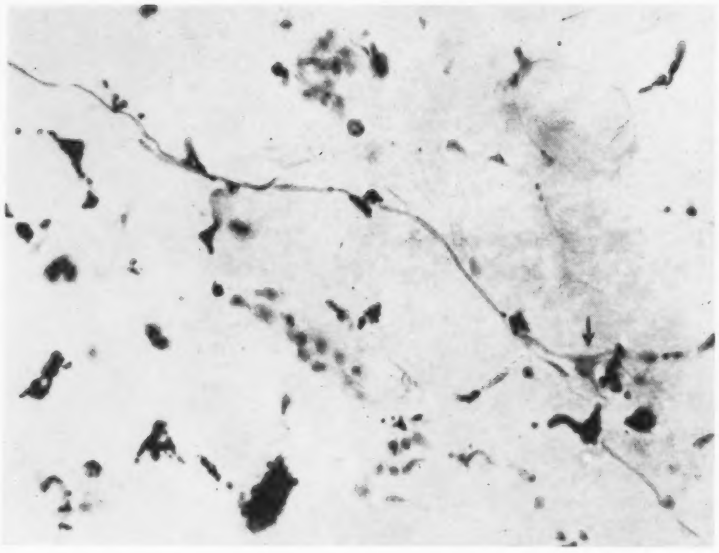

Fig. 10. Terminal branch and cell-like broadening of fibre (indicated by arrow) when branching in loose connective tissue dorsally to the kidney. Microphotograph, 340 ×.

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from the roots but also by the peripheral distribution of the fibres. The area of termination usually covers the space of several spinal roots (Fig. 1). In addition, it seems that neighbouring fibres are often united by longitudinal branches which run in the horizontal connective tissue membrane. In many cases these connecting branches are thick and run without any sign of interruption between the autonomic fibres which emerge from two separate dorsal roots. It has not been possible to discern whether this type of longitudinal connections is a general phenomenon owing to the fact that the horizontal connective tissue membrane is heavily laden with pigment cells among which usually only the thick fibres can be traced with certainty. Further, it has been observed only between autonomic fibres which emerge from dorsal roots. In the cases observed no peripheral cell bodies are present in these connections. It is evident that this peculiar type of connection makes the innervational units still larger and obscures the segmental arrangement still more.

In one single case a peripheral neuron has been observed which seems to have a different character from that of the peripheral neurons of terminal sympathetic plexuses (Fig. 11). (1) The cell is situated close to the ventral side of the notochord in the connective tissue between the aorta dorsalis and the left cardinal vein. (2) The cell is connected with the ventral ramus of a dorsal root by a thin preganglionic fibre. (3) Postganglionic nerve processes from the cell run between the aorta and the cardinal vein, ine anteriorly and one posteriorly. (4) The size of the cell body is only slightly less than that of

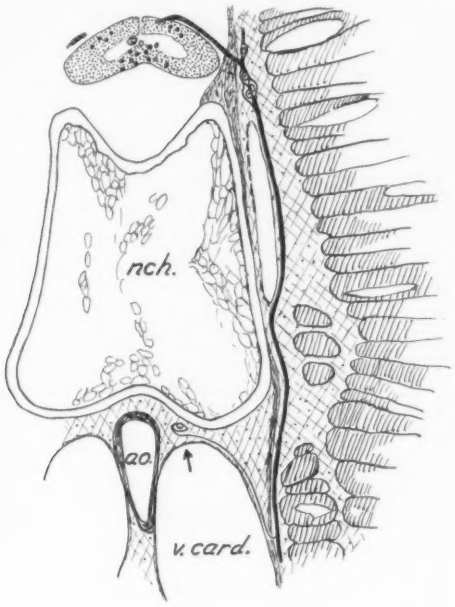

Fig. 11. Position and relation to ventral ramus of a dorsal root of a peripheral ganglion cell. Drawing, about 45 ×. ao. aorta, nch. notochord, v.card. left vena cardinalis posterior.

spinal ganglion cells. The smallest diameter of this ovoid cell is about 16 microns and that of the nucleus about 10 microns. The same measurements for spinal ganglion cells are 18—25 and 11—14 microns, respectively. (5) This large ganglion cell is accompanied by three or four small cells which seem to be identical with chromaffin cells (see p. 266 ff.). The peripheral cells situated in the cloacal region (cf. below) may be of the same type as this large cell.

The terminal connections of the longitudinal fibres of this large ganglion cell could not be observed. The fibres could be traced in the connective tissue between the aorta, the cardinal vein and the notochord beyond the consecutive dorsal root anteriorly and not quite as far caudally. If such neurons were common in the same position in other portions of the body there is not the slightest doubt that they would have attracted attention in the microscope. Thus it seems reasonable to conclude that they are very rare.

Often the branches of the sympathetic fibres which emerge from the horizontal membrane run along small arteries which extend to and enter the kidney parenchyma (Fig. 6). In other cases the fibres do not run along arteries but traverse the adipose tissue dorsally to the kidney before entering the kidney parenchyma (Fig. 7). Branches also seem to terminate within this adipose tissue. Other branches enter the gonad where they distribute themselves in the connective tissue net-work.

FREUD (1879) seems to have been the first to notice that there are considerable variations in the thickness of fibres of dorsal roots and that there are

usually one or a few comparatively very thick fibres among the normal, thin ones. FREUD pointed out that these thick fibres, and a number of thin ones too, pass through the spinal ganglia without contact with the ganglion cells. However, the technique of FREUD did not allow of a study of the peripheral distribution of the spinal nerves, and his statement to the effect that there is a sympathetic ventral nerve branch from each spinal nerve is based only on analogy with facts known from higher vertebrates.

In a previous paper FREUD (1877, and earlier KUTSCHIN, cf. FREUD 1878) had mentioned that there are fibres in the dorsal roots which emerge from the peculiar type of cells in the spinal cord which were originally described in Myxine by MÜLLER (1839) and termed "Hinterzellen" by REISSNER (1860) and LANGERHANS (1873). These cells of cyclostomes have been regarded as homologous with Rohon-Beard cells of other vertebrates (fishes, amphibians, cf. WHITING 1948). However, FREUD was unable to trace the origin within the spinal cord of the thick fibres of the dorsal roots. TRETJAKOFF (1909) completely denied that dorsal root fibres could originate in the said cells in the spinal cord. According to him all fibres of the dorsal roots belonged to the spinal ganglion cells. However, KOLMER (1905) and BECCARI (1909) pictured and described dorsal root fibres originating in the "Hinterzellen" and, thus, confirmed the statements of FREUD.

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It has already been mentioned that in the present material the thick autonomic fibres, when present, are easily distinguished from the rest of the fibres of a dorsal spinal root. As a consequence it is possible to trace these thick fibres centrally through the spinal ganglia and to see that there is no contact with spinal ganglion cells. In some cases it has been possible also to follow these fibres to their origin in one of the dorsal cells of the spinal cord. Usually the thick fibres run for a considerable distance in the white matter of the spinal cord as noticed by FREUD. He was, however, unable to observe the actual connection with a cell body. In the present material it has, thus, been possible to follow up visceral fibres from the periphery to the origination in cells in the spinal cord.

WHITING (1948) states that the Rohon-Beard cells of young lamprey larvae have somatic-sensory processes. As there can be no doubt that the thick fibres described above have an autonomic function there are consequently also visceral dorsal cells. BECCARI (1909) distinguished four different types of these cells. It is possible that different functions may be ascribed to different types of these cells but this possibility has not been examined in this work.

2. THE INNERVATION OF CHROMAFFIN ELEMENTS

The distribution of chromaffin (adrenal) elements in lampreys has been described by GIACOMINI (1902) and GASKELL (1912). The chromaffin cells form large units in the walls of the Cuvieran duct, the anterior parts of the posterior

cardinal veins, the adjacent parts of the vessels from the head, and along the segmental vessels throughout the length of the body. GASKELL (1912) pointed out that the chromaffin cells are connected with the segmental veins and with the nerve fibres of the dorsal roots rather than with the segmental arteries. The ventral branch of the segmental vein is usually more distant from the segmental artery and the spinal nerve than the dorsal branch. In these cases the chromaffin tissue is related in the first place to the venous wall. It seems that these segmental chromaffin elements were observed already by FREUD (1879) although he interpreted them as mulipolar ganglion cells. Also TRETJAKOFF (1927) most probably referred to the same elements when he notified the presence of sympathetic ganglion cells in the walls of the large blood vessels in the trunk. Smaller aggregations of chromaffin cells are present in all parts of the heart (AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND 1956), in the wall of the anterior cardinal veins and the median (ventral) jugular vein, in the liver, in the anterior part of the intestine, etc.

The extent of the chromaffin tissue in the walls of the segmental veins varies considerably. In some cases the cells form a more or less complete lining of the wall of the vein (cf. Fig. 4). In other cases only scattered cells are present which are more on less distinctly connected by cell processes with each other. When the number of cells is large it is usually easy to observe that one or two of the thick fibres which pass the spinal ganglion without a contact with the spinal ganglion cells ramify in the immediate vicinity of the chromaffin cell layer. Very often this takes place in the angle between the dorsal and the ventral ramus of the spinal nerve, just distally to the spinal ganglion. These cases are very similar to the ramifications "in adipose tissue" discovered and pictured by TRETJAKOFF (1927, Fig. 25) and they are probably identical in part at least. In other places the ramifications take place more distally somewhere along the ventral or dorsal ramus of the spinal nerve (Fig. 12). When these thick fibres start to ramify they usually give off many branches. The thickness of the fibre diminishes because of this very suddenly and the end ramifications are very delicate.

The largest accumulation of chromaffin elements which is present in the body of lampreys is situated in the walls of the anterior part of the posterior cardinal veins. This accumulation is particularly dense in the vicinity of the arteria coeliaca (A. mesenterica) which traverses the cavity of the anterior, united portion of the two venae mentioned ("the Cuvieran duct"). This region is innervated by large nerve fibres which—in the specimen pictured in Fig. 1 b —emerge from dorsal roots on the right side of the animal (not seen in the picture) and by a small nerve, composed of several rather thin fibres, which comes from a dorsal root on the left side (Fig. 1 b). This small nerve terminates (Fig. 13) in branches which spread in the connective tissue situated between the liver and the alimentary canal, ventrally, the anterior portions of

Fig. 12. A thin nerve fibre (indicated by arrow) terminating in chromaffin tissue. The notochordal sheath to the right. Photomicrograph, 450 ×.

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the posterior cardinal veins, laterally, and the dorsal aorta and the notochord, dorsally (an anterior remnant of a mesenterium, cf. MARINELLI & STRENGER 1954). One single fibre of this nerve has been observed to form a loop running laterally and back into the horizontal connective tissue membrane. It is possible that this nerve may also innervate structures in the vicinity such as the arteries destined to the liver and pancreas.

The mesenterial ramifications are distributed to the chromaffin cells situated in the mesenterium. Many of these cells are located at some distance from the main layer along the walls of the vessels and completely embedded in connective tissue. These cells are connected with the cells of the layer in the venous walls by thin cell processes (Fig. 13).

When the chromaffin cells form layers in the walls of segmental veins they are flattened towards the walls of the veins. The cells have a more or less polyhedral shape as seen in surface sections (Fig. 4). They border closely on each other and most of them do not have prominent cell processes. In the place where the arteria coeliaca traverses the lumen of the united cardinal veins (sometimes termed the Cuvieran duct or regarded as the dorsal part of sinus venosus), the exterior surface of the arteria which is turned towards the venous lacuna is more or less covered with chromaffin cells which form a layer so dense as to be reminiscent of a simple columnar epithelium (Fig. 14). These cells seem to lack every trace of cell processes. In most cases, however, the chromaffin cells are sparser and the shape is, then, very similar to that of a multipolar ganglion cell (cf. Fig. 13). The shape of the cells in the heart has been described earlier (AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND 1956) and is also similar to that of multipolar cells.

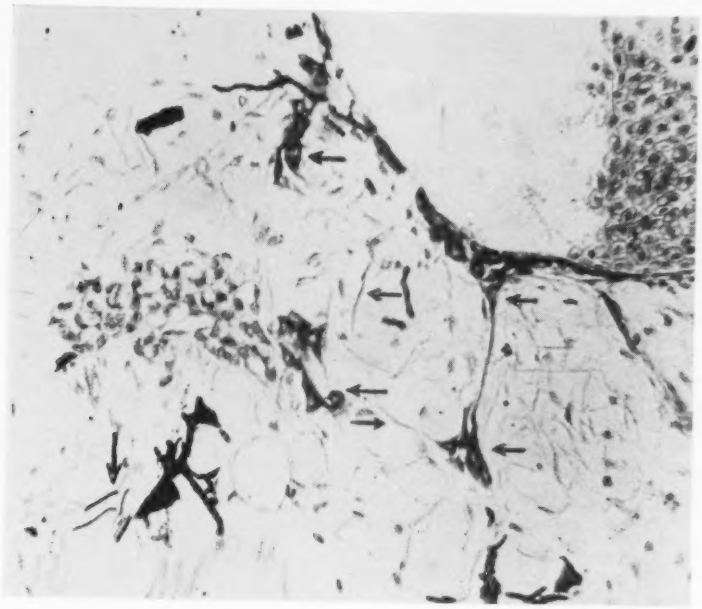

Fig. 13. Chromaffin cells-ganglion cells in the short broad remnant of a mesenterium dorsally to the liver in an adult specimen. The small nerve indicated by an arrow in Fig. 1 b is indicated by a vertical arrow to the left. Chromaffin cells-ganglion cells and connecting fibres indicated by horizontal arrows. Dorsally to the nerve and the large pigment cell an accumulation of interrenal tissue. Vena cardinalis in the upper right corner, partly lined with chromaffin cells. Photomicrograph, 340 ×.

As mentioned, cells which are very likely identical with these chromaffin cells have been regarded as ganglion cells by some authors (e.g. FREUD 1878, TRETJAKOFF 1927). In many cases the chromaffin cells of the trunk really have the shape of ganglion cells with slender cell processes and are situated without the intimate contact with venous lumina which would be expected of typical adrenal cells. From this type of chromaffin cells all intergradations are represented to the other extreme represented by the epithelium-like cells outside the wall of arteria coeliaca. In the case of the chromaffin cells of the heart the view has been expressed that these cells form a primitive type of sympathetic (adrenergic) ganglion cells which together with a small number of larger ganglion cells form the innervational system of the heart (AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND 1956). This view may be extended to apply also to the rest of the chromaffin cells of the body.

In the heart the chromaffin elements are probably innervated by the heart branch of the vagus nerve and the large type of ganglion cells which is present in the heart (cf. pp. 256—257) is most probably interpolated in this system. There may also be a sympathetic influence via the chromaffin system of the walls of the large veins which are continuous with that of the walls of the sinus

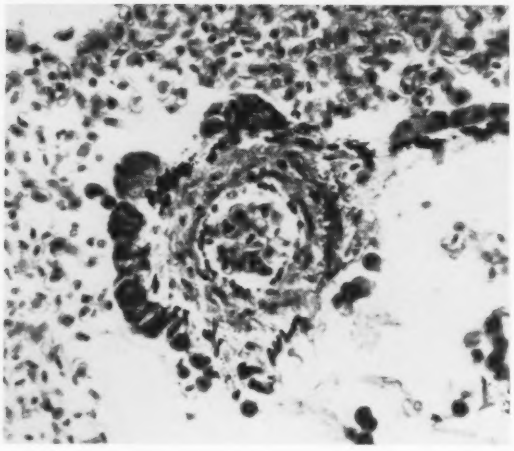

Fig. 14. Arteria coeliaca cross-sectioned. The outside of the wall borders on the lumen of the anterior united portion of the two venae cardinales. The venous wall is lined with chromaffin cells reminiscent of columnar epithelium cells. Photomicrograph, 340 ×.

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venosus (cf. AUGUSTINSSON, FÄNGE, JOHNELS, and ÖSTLUND 1956). Fibres from the vagus intestinalis are also distributed to the immediate vicinity of groups of chromaffin cells in the liver parenchyma and in the spiral valve of the intestine. All other chromaffin elements of the trunk region seem to be innervated by preganglionic fibres from spinal nerves as has been described above.

GIACOMINI (1902) stated that what were considered to be sympathetic ganglia by JULIN (1887) were most probably nodules of interrenal tissue. Considering the methods employed by JULIN and the positions of the "sympathetic ganglia" as indicated in pictures and text by JULIN this seems to be a correct conclusion. It should be stressed, however, that interrenal cell groups are very often in contact with chromaffin elements in lampreys (cf. Fig. 13). The statements made by KRAUSE (1923, p. 858) regarding the adrenal and interrenal organs are in some respects highly puzzling and probably mainly erroneous.

3. THE INNERVATION OF THE CLOACAL REGION

The rectum of the adult lamprey opens into the anterior portion of the cloaca. In the caudal part the urogenital papilla is situated. On the tip of the papilla the urogenital sinus opens. A short distance from this mouth of the urogenital sinus the genital pore opens into the excretory duct. All the ducts have a fairly strong layer of smooth musculature in the walls. Also the rectum has a strong muscle layer as compared with the rest of the intestine. The space dorsal to the rectum and the cloaca and between the excretory ducts is filled with a loose connective tissue and the whole is surrounded dorsally and laterally by a strong cap of striated muscles (nodus muscularis cloacalis, MARINELLI & STRENGER 1954, cf. also DOHRN 1888, LICKTEIG 1913, KRAUSE 1923). These striated muscles and the genital pore are missing in the larva. These structures

are formed during the metamorphosis. The formation of the musculature takes place from a sort of primordium which is present in the larva and develops during the metamorphosis in a way reminiscent of the formation of the musculus subocularis described by DAMAS (1935).

The fact that there is a particularly strong innervation of the cloacal region has attracted attention earlier (DOHRN 1888, KRAUSE 1923, TRETJAKOFF 1927). A number of the cloacal nerves enter the posterior end of the gut and are distributed into the comparatively very strong smooth musculature of the rectum. However, it is not the digestive tract which is the destination of most of these nerves—at least not in the first place—as has been anticipated earlier (cf. NICOL 1952) but the cloacal apparatus.

The innervation of the cloacal apparatus is peculiar in several respects in comparison with the autonomic innervation of viscera in other regions of the trunk. Thus, there are (1) a large number of roots—dorsal as well as ventral ones—which take part in the innervation (in the cases observed about 15 roots on each side). (2) With certain exceptions (cf. below) no thick fibres of the dorsal roots take part in this innervation. Instead there are usually many thin fibres, forming small nerves, which arise in the roots and are concentrated towards the cloaca. Further, (3) peripheral ganglion cells of a particular type play an important role in the innervation in the larva as well as in the adult. However, many of these peripheral neurons and several of the nerves must develop during the metamorphosis as there is a larger number of neural elements in the adult than in the larval organization.

To a large extent, at least, the nervous apparatus in the adult seems to be concerned with the innervation of the cloacal musculature proper which is partly striated. This part is, as mentioned, missing in the larva. However, a considerable number of fibres are distributed towards the wall of the intestine close to its caudal extremity. Also the ureter is innervated by nerve branches which enter the wall of the excretory duct near its posterior extremity in the cloacal region. The nerves which enter the walls of the intestine and the ureter in this way arise from roots which are situated anteriorly to the cloacal portion of the body. In fact, the roots from which these intestinal and uretral branches arise are always the most anterior of all the roots which distribute nerves towards the cloacal region (cf. Figs. 15 and 18). In all the cases observed the intestinal and uretral branches emerge exclusively from dorsal roots.

All the small nerves which proceed to the intestine or the ureter follow a very characteristic course (Fig. 16). The visceral fibres either soon branch off from the ventral ramus of the dorsal root and proceed in a ventro-caudal direction or else proceed together with the other fibres of the ventral ramus of the root, in both cases until a very ventral position in the body is attained. So far the visceral fibres in conformity with the other nerve fibres of the ramus run in the loose connective tissue between the muscular wall of the body and the

Fig. 15. The innervation of the intestine and the ureter in the cloacal region. Reconstruction, left side view, 18 ×. The termination area of intestinal branches indicated by a vertical arrow, the ureter fibres by oblique arrows. Broken lines are thick fibres to the kidney parenchyma. Ventral roots and pertaining nerves dotted. Small circles indicate ganglion cells situated outside the cloacal apparatus.

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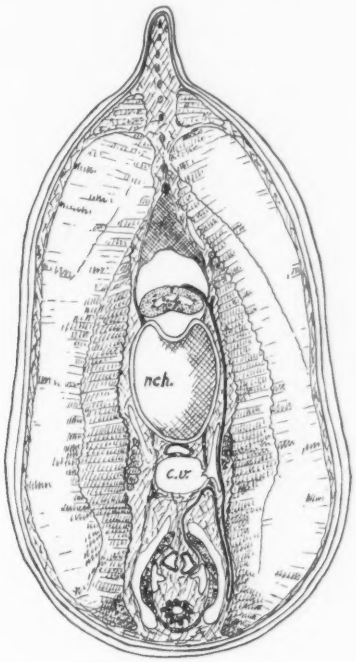

Fig. 16. The course of cloacal nerves. The cloacal apparatus with the rectum ventrally, the ureters (thick lines), the coeomoducts (thin lines), and the striated muscle cap. Drawing, 16 ×. c.v. cardinal vein, nch. notochord.

parietal membrane but here the visceral fibres enter into the laminated connective tissue of the parietal membrane. From here the nerves proceed within the dense connective tissue of the parietal membrane in a mainly caudo-dorsal direction until the place is reached where the horizontal membrane unites with the parietal one. At this place which may be at a considerable distance caudally from the start in the spinal root (cf. Fig. 15) the nerve enters into the viscera and runs in a ventro-caudal direction in the mesenterium to the vicinity of the cloaca. Here, at least one of the main branches of the nerve turns abruptly in the opposite direction, enters the wall of the rectum or the ureter from the caudal end and runs, from this place on, in a cranial direction. In addition to these rectal and uretral branches other branches are distributed to the parts of the alimentary canal, the excretory duct, and the coelomoduct which are within the cloacal region. There are three to five such visceral nerves which emerge from as many dorsal roots.

The fibres directed to the rectum soon separate from each other and ramify in the smooth muscle layer. On the whole they do not reach very far anteriorly (cf. Fig. 15). In the ureters the innervation is stronger and more than one root sends nerves towards them. Several more or less distinct nerves, composed of very fine fibres, reach very far anteriorly along the excretory ducts (cf. Fig. 15).

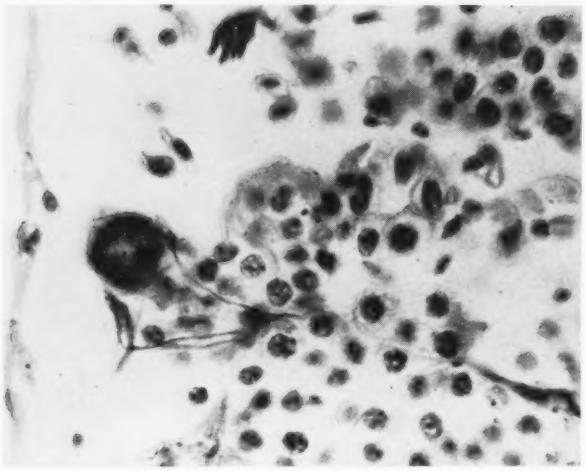

Fig. 17. Ganglion cell of the cloacal type in the kidney parenchyma. A number of very thin (postganglionic?) fibres are also seen. Above a dark bundle of preganglionic fibres. Microphotograph, 680 ×.

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In the cases described above it is exclusively a question of preganglionic fibres. In addition to these fibres the nerves to the rectum and ureters contain fibres which seem to emerge from the vicinity of the cloacal region and which are probably postganglionic. These fibres originate in the ganglion cell plexus which is distributed below the muscle cap which is formed by striated musculature in the cloacal region. There are several small nerves which seem to originate in this region and enter the walls of the rectum and the ureter (indicated in Fig. 15). In addition, the larger branches of the nerves within the ureters and the rectum contain a few ganglion cells (Fig. 17) of the same type as these in the cloacal region which are—particularly in the case of the ureters —often situated at a considerable distance from the latter region. In the caudal part of the alimentary canal there are, also, other ganglion cells which are typical of the nerve plexus of that organ (Fig. 3).

The rectal and uretal preganglionic nerves originate in the most anterior of the roots which send branches towards the cloacal region. In these nerves there are, usually, one or two thick fibres of the type which distribute their branches to the parenchyma of the kidneys (indicated by broken lines in Fig. 15). The type of probably vasomotor innervation of this organ which is found in the main part of the trunk region is, thus, present also in the posterior portion.

The rest of the nerves which enter the cloacal region arise from dorsal as well as from ventral roots (Fig. 18). The cloacal branches from the different dorsal and ventral roots often unite into larger nerves which run to the area of termination among the ganglion cells and smooth muscles below the striated muscle cap and in the tissue of the latter muscle. From the ventral roots there arise

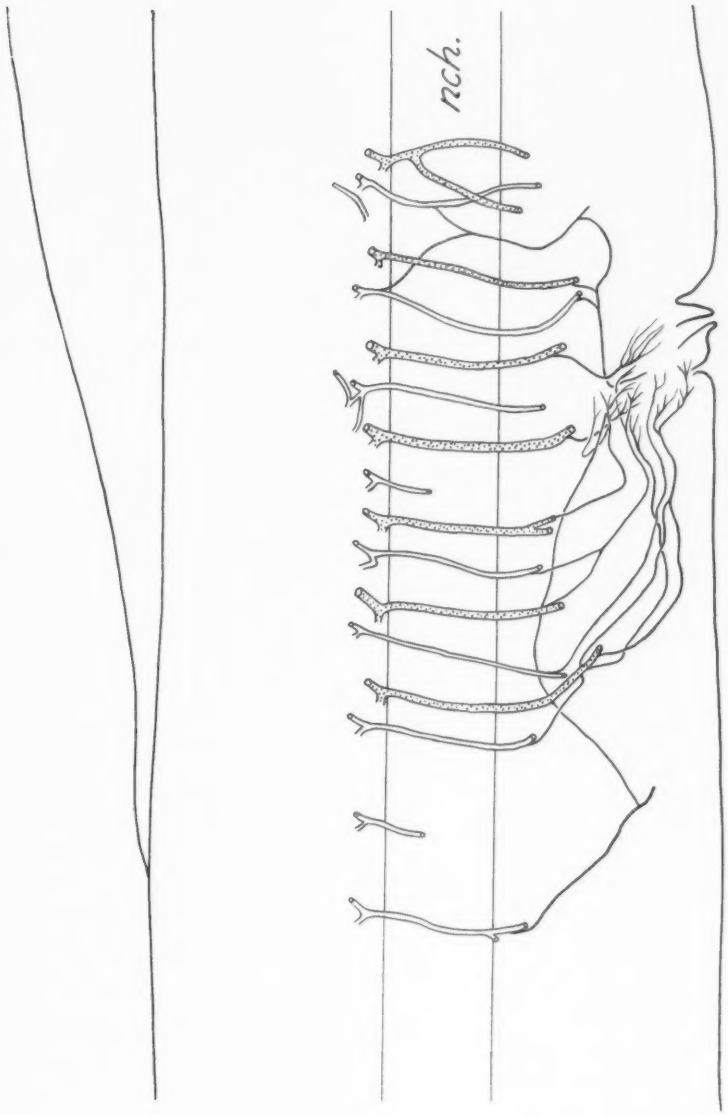

Fig. 18. The innervation of the cloacal apparatus. See also Fig. 15. Reconstruction, left view. 18 ×. Ventral roots and pertaining rami dotted. Irregularities in the arrangement of spinal roots and nerves indicated.

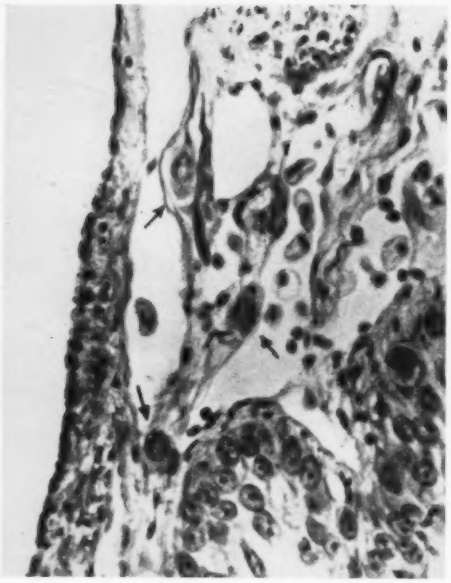

Fig. 19. Fibres and ganglion cells (indicated by arrows) in the cloacal region of the larva. Smooth muscle layer to the left, rectal epithelium in the lower right corner. Microphotograph, 460 ×.

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a large number of thick fibres which have the same structure as the typical somatic motor fibres. These thick fibres—to a large extent at least—run to the striated muscles of the cloacal muscle cap. This cap is probably to be regarded as a separated and specialized part of the segmental somatic muscle (cf. DOHRN 1888) and consequently the pertaining fibres can hardly be autonomic. However, in the larva where these striated muscles are not yet developed fibres of a considerable thickness are seen in the cloacal region (cf. Fig. 19). It is possible that the cap muscles are under the influence of the autonomic system, too, as the ganglion cells are often very intimately connected to the interior side of the cap musculature. Apart from the thick fibres mentioned thin fibres also emerge from ventral roots. These fibres run in the nerves to the cloaca and seem to be autonomic.

The rectum and particularly the posterior parts of the ureters and the body cavity conducting into the genital pores (the coelomoducts) have a comparatively strong layer of smooth muscles. In the loose connective tissue around these structures, particularly dorsally to them below the cap, a large number of comparatively large ganglion cells is distributed (Fig. 20). They are located in the meshes of a complicated net-work of preganglionic and postganglionic fibres. One or two of the nerves which are directed towards this area contain a ganglion, composed of several ganglion cells, which is situated laterally to the cloacal apparatus in loose connective tissue (Fig. 21). These ganglia are present both in the adult and in the larva. However, the number of ganglion

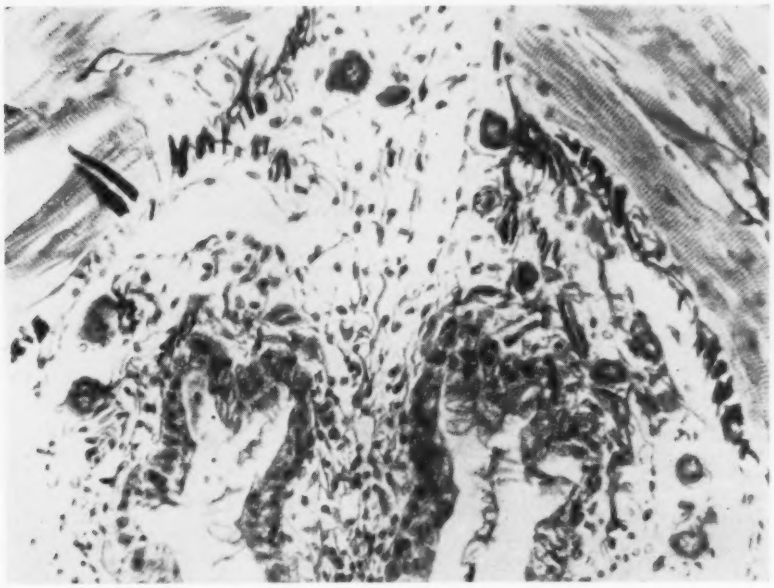

Fig. 20. Fibres of various calibres and ganglion cells in the cloacal apparatus below the striated nodus muscularis cloacalis. The two ureters below in the picture. Adult. Microphotograph, 340 ×.

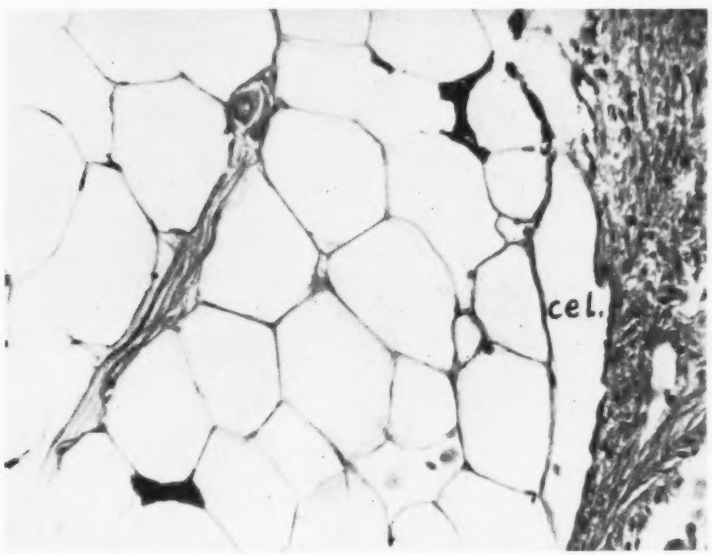

Fig. 21. Cloacal nerve with small ganglion. A part of the cloacal apparatus seen to the right. Larva. Microphotograph, 340 ×. cel. coelom.

cells below the cap seems to increase during the metamorphosis. These ganglion cells seem to be bipolar or multipolar. They are larger than the ganglion cells of the intestine but smaller than the spinal ganglion cells.

The ganglion cells of the cloacal type which are situated in the posterior parts of the alimentary canal and the ureter may function in coordinating the reactions in these organs with the reactions of the cloacal apparatus proper. Assumably, this function is, in the adult, to a large extent related to the act of propagation and the discharge of the genital products.

The sensory innervation of the epidermis is very strong in the cloacal region. Particularly densely arranged are the nerve endings in the body wall epithelium just caudally to the cloaca. Many of the nerves to this area which originate in the spinal nerves run together with fibres which run to the cloacal region. It seems possible that also a number of the fibres distributed to the cloacal apparatus are sensory. No difference between the sexes has been observed in the organization of the cloacal innervation.

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V. DISCUSSION

Goodrich (1930) subdivided the autonomic nervous system in gnathostomes into (1) a dorsal-root system and (2) a ventral-root system. However, he considered the outflow of autonomic (preganglionic) fibres to be restricted exclusively to dorsal roots in lower vertebrates (Amphioxus and lampreys) and believed this connection to be primitive. Because of this Goodrich (1930, pp. 784 ff.) formulated the following problem: "it remains to be explained how in the Gnathostomes sympathetic neurons — — — acquired a connection with the central nervous system by means of preganglionic fibres issuing through the ventral roots". This problem has been discussed also by Nicol (1952).

However, Ayers (1921) claimed that there is an outflow of autonomic fibres not only from the dorsal but also from the ventral roots, to the sympathetic nerve plexus in the aortic wall, in Amphioxus. From Nicol (1952, pp. 32 and 33) the reader may get the impression that Ayers in a later work (1930) found that also in lampreys autonomic fibres emerge from ventral roots. However, Ayers in this work dealt with myxinids exclusively. Further, he indicated only in a drawing that a "splancnic nerve" may emerge from a motor ramus, a fact which is not discussed in the text. In 1937 Goodrich still considered this question to be unsettled as regards myxinids (cf. also Nicol 1952) and there is, in the literature, no other evidence than that used by Goodrich regarding lampreys.

In the present investigation the emergence of autonomic (preganglionic) fibres from ventral as well as dorsal roots has been observed to be a general feature in the trunk region in adult lampreys. In the cloacal region real nerves —the autonomic nature of which is in some cases obscure—emerge from both

dorsal and ventral roots. In view of these facts it seems reasonable to conclude that the common ancesters of cyclostomes and gnathostomes also had preganglionic fibres emerging from both dorsal and ventral roots. The particular type of autonomic system with a different outflow of autonomic elements from the central nervous system in different regions of the body which is found in gnathostomes may have evolved from such a state.

It seems that lampreys lack structures which correspond to a commissural sympathetic chain in the trunk and tail (cf. however below). Except in the cloacal region the visceral preganglionic fibres terminate in the terminal plexus without the interpolation of postganglionic neurons aggregated in ganglia. This is the case with all autonomic fibres in Amphioxus. There is a widespread emergence of visceral fibres in lampreys but the outflow is not as regular as in Amphioxus. Lampreys are said to possess at least a tendency towards a commissural sympathetic chain in the head region (JOHNSTON 1905, TRETJAKOFF 1927) and have a peculiar organization of the autonomic innervation of the cloacal region. There is, thus, a regional differentiation in these animals which is absent in Amphioxus although it is much less elaborate than in gnathostomes.

The formation of sympathetic ganglia is extremely imperfect in lampreys. The peripheral cell bodies are diffusely distributed in the terminal plexuses of the viscera. The chromaffin cells (which may represent postganglionic neurons) and the arterial walls seem to be innervated by preganglionic fibres. In selachians (YOUNG 1933) and chimaeroids (NICOL 1950) sympathetic ganglia are formed but structures representing connectives and commissures are very tenuous. In teleosts, however, the typical vertebrate sympathetic chain with segmental ganglia, connectives and commissures is present.

In higher vertebrates, including chimaeroids and selachians, there is a sympathetic innervation of the urogenital ducts. This is also the case in lampreys. In lampreys there is, also, an autonomic innervation of the rectal portion of the alimentary canal with a system of postganglionic neurons involved. This is interesting in view of the fact that in selachians and chimaeroids the rectal part of the abdominal canal receives an inflow of preganglionic fibres which terminate without the interpolation of postganglionic neurons (YOUNG 1933, NICOL 1950). It seems probable that lampreys reveal the more primitive condition in this respect.

That lampreys have no mesenterium which connects the intestine with the body in the trunk region can hardly be considered to be primitive. The loss of the main part of a mesenterium may be the explanation of the fact that there are central connections of the autonomic system only at the ends of the alimentary canal. Because of this it could be postulated that the innervation was secondarily largely taken over by the vagus intestinalis which would explain its prolonged caudal extension, demonstrated in the present investigation. However, in Myxine BRANDT (1922) confirmed the observations of MÜLLER (1839) that

there is a fusion of the two intestinal branches of the vagus nerve into a single intestinal nerve which runs in the dorsal surface of the gut to its caudal extremity. Myxine has no spiral valve and this may be the explanation of the superficial position of the vagus intestinalis in Myxine. BRANDT did not find any other source of autonomic fibres to the alimentary canal than the vagus. Myxine, in contrast to Petromyzon, has an extensive mesenterium.

BRANDT was impressed by the large number of nerve fibres at the caudal extremity of the intestine in Myxine but he considered these to be the caudal extension of the vagus nerve. However, it seems possible that this rich supply of fibres really may indicate an inflow of autonomic fibres at the caudal end of the alimentary canal also in Myxine. In any case there is a remarkable similarity between lampreys and hagfishes in (1) the dominating vagus innervation of the intestine and in (2) the absence of fibres from spinal nerves to the alimentary canal along most of its extension.

The prolonged caudal extension of the vagus intestinalis is mostly believed to be secondary in higher groups of vertebrates (IHLE etc. 1927, CORDS 1929, HALLER v. HALLERSTEIN 1934, NICOL 1952). The primitive type of an autonomic nervous system is assumed to have consisted of segmentally arranged preganglionic fibres which reach a terminal network of neurons in the end-organs, and among them the intestine (cf. NICOL 1952).

It is interesting to notice, in this connection, that in both the groups of cyclostomes the vagus forms the dominant innervation of the alimentary canal. Therefore it may be concluded—if the alimentary canal took part in the above mentioned primitive organization—that the caudal extension of the vagus intestinalis most probably must have taken place already in the common ancestors of cyclostomes and gnathostomes. In consequence the evolution of the autonomic nervous system in the various groups of gnathostomes may have involved a reduction or at least a specialization instead of an augmentation of the vagus intestinalis system. Simultaneously and possibly in connection with this process a differentiation (with a widespread interpolation of postganglionic fibres?) of the sympathetic system probably took place. Although a double innervation of the gut is present in higher vertebrates the intramural nerve cells are believed to be postganglionic neurons of the vagus nerve exclusively (cf. KUNTZ 1947, NICOL 1952). Also this tallies well with the idea of an original dominance of the vagus intestinalis in the ancestors of vertebrates.

Apart from the innervation of the intestine the structure of the autonomic nervous system of lampreys is in good accordance with the idea of the primitive organization of this system, which was mentioned above. The organization of the sympathetic nervous system of lampreys is in several respects intermediate between that of Amphioxus and that of gnathostomes.

In one case a peripheral neuron has been observed which in many respects is similar to and in the same position as a postganglionic neuron in the sym-

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pathetic trunk of gnathostomes (cf. p. 264). Although several thousands of sections have been scrutinized for similar cells in approximately the same position not one more has been detected. The present data permit the conclusion that such cells are very rare. But at the same time it seems that they do exist, and it is very tempting to regard them as homologous with the sympathetic trunk neurons of gnathostomes. The similarity is accentuated by the presence in the immediate vicinity of this particular neuron of a small number of cells of the particular chromaffin type which is present in lampreys (see below). The ganglion cells of the cloacal region seem to be of the same type and here the system with interpolated neurons is, thus, established. Small peripheral cells similar to those in the enteric plexus have been observed in the connective tissue of the gonad and dorsally to the kidneys, but only in small numbers. A connection between these cells and the visceral nerve fibres has not been definitely demonstrated. They have not been observed in the arterial walls.

Nicol (1952, p. 23) pointed out that "it is noteworthy that a scattered suprarenal tissue is clearly differentiated from the sympathetic system in these animals". The question is, however, how far this differentiation has advanced in lampreys. As regards the heart the opinion has been expressed earlier that the chromaffin cells of this organ may form some kind of innervational system in spite of their chromaffin nature (Augustinsson, Fänge, Johnels, and Östlund 1956) although the larger type of ganglion cells which was also observed in the heart probably represents real postganglionic neurons of the vagus.

The innervation of the chromaffin elements takes place by preganglionic fibres. It seems, however, that this fibre innervation is very restricted and distributed only to a small minority of the cells which form the chromaffin tissue which often has a large extension (cf. above Ch. IV, 2). This seems to indicate that impulses may spread through the chromaffin cells. In many cases also the mutual arrangement of the cells is very similar to a conducting system of neurons (cf. e.g. Fig. 13), although this, in other cases, is not indicated (cf. Fig. 14). A differentiation among the chromaffin cells into what could be conducting cells and suprarenal cells has not been observed. But often the cells are extremely similar to small neurons. In fact skilled neurologists have earlier regarded the chromaffin cells of the segmental veins as nerve cells (e.g. Freud 1878, Tretjakoff 1927). In consequence it seems possible that the chromaffin cells of lampreys represent a primitive type of adrenal cell with great similarities to sympathetic (adrenergic) neurons. In gnathostomes the adrenal cells are considered to emerge ontogenetically from the same source as sympathetic neurons, from the neural crest (literature in Nicol 1952, Bachmann 1954, cf. however Kuntz 1947) which also gives rise to other elements, including the ganglion cells of the dorsal roots (cf. Hörstadius 1950). However, chromaffin cells which also have a function as neurons have earlier been observed only in invertebrates (J. F. Gaskell 1914, 1920, Hanström 1939).

On the other hand it seems justifiable to assume that the widespread occurrence of chromaffin elements in the trunk of lampreys may have restrained the distribution of typical sympathetic postganglionic elements. As is well known the chromaffin system (cf. e.g. CELESTINO DA COSTA 1936, NICOL 1952, BACHMANN 1954, LANDAU 1956) of higher gnathostomes has a larger distribution in the early ontogenetic stages than in the adults, and in lower gnathostomes the same system generally has a larger or at least a more scattered distribution than in higher gnathostomes. It is, thus, indicated that in the evolution of the chromaffin-sympathetic system in gnathostomes the chromaffin elements became diminished and specialized and the sympathetic elements were increased. The structure of the chromaffin-sympathetic system in lampreys with a widely spread chromaffin component and a weakly developed sympathetic component (particularly regarding peripheral neurons) agrees well with what could be expected of a primitive form.

No nerves have been observed in the aorta or the peculiar structures at the beginning of the segmental arteries in the aortic wall. On the other hand, nerves have been demonstrated to innervate the wall of segmental and other arteries. Apart from nerve fibres distributed to the chromaffin tissue situated in the walls of the veins no innervation has been observed in the venous walls. It seems possible that only the arteries are immediately controlled by nerves. The veins (together with other organs) may be influenced exclusively via the humoral mechanism of the chromaffin system.

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VI. SUMMARY

The autonomic nervous system of Lampetra planeri, larval as well as adult stages, has been studied with protargol techniques. The following main results have been obtained.

1. It has been demonstrated that the two intestinal branches of nervus vagus unite at the limit between the oesophagus and the intestine and run in the tissue of the spiral valve until close to the rectal portion of the alimentary canal.

2. There is no innervation of the alimentary canal except at the anterior and posterior ends.

3. The gonad and the kidneys are innervated by branches, mostly single nerve fibres, which emerge from spiral roots. There are no peripheral neurons aggregated to ganglia between these fibres and the terminal plexus.

4. Visceral fibres emerge from dorsal as well as from ventral roots of the spinal nerves.

5. A number of visceral fibres of dorsal roots have been traced to their origin in cells within the spinal cord.

6. The innervation of the chromaffin cells by preganglionic fibres is described. The nature of the chromaffin elements is discussed and the possibility that the chromaffin cells of the lamprey may form a primitive type of suprarenal cell with great similarities to a sympathetic (adrenergic) neuron is pointed out.

7. The innervation of the cloacal region is described. There are nerves to the rectum, to the ureters, and to the musculature of the cloacal apparatus. In connection with this apparatus in the first place, a large number of postganglionic neurons are interpolated.

8. A comparison with the autonomic nervous systems of Amphioxus, Myxine, and gnathostomes is made. The opinion is expressed that the prolonged caudal extension of the vagus intestinalis which is present in both Petromyzon and Myxine was also present in the common ancestors of cyclostomes and gnathostomes and that, consequently, the prolonged caudal extension of the vagus intestinalis is not a secondary phenomenon in vertebrates.

9. The outflow of autonomic fibres in dorsal as well as in ventral roots in lampreys can easily explain the conditions in this respect in gnathostomes.

10. There is a regional differentiation of the autonomic system in lampreys which is absent in Amphioxus but it is less elaborate than in gnathostomes. There are postganglionic neurons of a certain type in the cloacal region, and very rarely also in the trunk. This recalls the system in gnathostomes. On the other hand the kidneys and the gonad have no postganglionic neurons aggregated in ganglia and in this respect lampreys are similar to Amphioxus. Thus, the autonomic system of lampreys is, in certain respects, intermediate between that of Amphioxus and that of gnathostomes.

11. The walls of arteries have been demonstrated to be innervated by preganglionic fibres. The veins may be influenced by the effect of the chromaffin system exclusively.

VII. LITERATURE

Agduhr, E. 1930. Einige Beiträge zur Ag.-Technik der Stückinprägnierung der Nerven. Anat. Anz. 69.

Ariens Kappers, C. U., Huber, C. G., and Crosby, E. 1936. The comparative anatomy of the nervous system of vertebrates including man. I—II. New York.

Augustinsson, K.-B., Fänge, R., Johnels, A. G., and Östlund, E. 1956. Histological, physiological and biochemical studies on the heart of two cyclostomes, hagfish (Myxine) and lamprey (Lampetra). J. Physiol. 131.

Ayers. H. 1921. Ventral spinal nerves in Amphioxus. J. Comp. Neur. 33.

— 1930. Amphioxine nerve in myxonoids. J. Comp. Neur. 50.

Bachmann, R. 1954. Die Nebenniere. In: Möllendorf, W. v., und Bargmann, W. Handb. d. mikr. Anat. d. Menschen. Berlin, Göttingen, Heidelberg.

BECCARI, N. 1909. La cellule dorsali o posteriori dei ciclostomi. Ricerche nel Petromyzon marinus. Monit. Zoool. Ital. 20.

BODIAN, D. 1936. A new method for staining nerve fibres and nerve endings in mounted paraffin sections. Anat. Rec. 65.

— 1937. The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anat. Rec. 69.

BOENIG, H. 1927. Studien zur Morphologie und Entwicklungsgeschichte des Pancreas beim Bachneunauge (Lampetra/Petromyzon/planeri). Zschr. Mikr.-Anat. Forsch. 8.

BRANDT, W. 1922. Das Darmnervensystem von Myxine glutinosa. Zschr. Anat. Entw.-gesch. 65.

CELESTINO DA COSTA, A. 1936. Nouvelles recherches sur le développement des paraganglia chez certains cheiroptères de la famille des Vespetilionidés. Arch. portug. sc. biol. (Porto) 5.

CORDS, E. 1929. Die Kopfnerven der Petromyzonten. Zschr. Anat. Entw.-gesch. 89.

DAMAS, H. 1935. Contribution à l'étude de la métamorphose de la tête de la lamproie. Arch. Biol. 46.

DOHRN, A. 1886. Studien zur Urgeschichte des Wirbeltierkörpers. IX. Die Bedeutung der unpaaren Flosse für die Beurtheilung der genealogischen Stellung der Tunicata und des Amphioxus, und die Reste der Beckenflosse bei Petromyzon. Mittheil. Zool. Stat. Napoli 6.

— 1888. Studien zur Urgeschichte des Wirbeltierkörpers. XIII. Über Nerven und Gefässe bei Ammocoetes und Petromyzon planeri. Mittheil. Zool. Stat. Napoli. 8.

FAVARO, G. 1908. Pisces. In: Bronn's Klassen und Ordnungen etc. Bd. 6, Pt. 6, 13, I.

FREUD, S. 1877. Über den Ursprung der hinteren Nervenwurzeln im Rückenmark vom Ammocoetes (Petromyzon planeri). Sitz.-ber. K. Akad. Wiss. Wien. 75.

— 1878. Über Spinalgangligen und Rückenmark des Petromyzon. Sitz.-ber. K. Akad. Wiss. Wien. 78.

FUSARI, R. 1907 a. Contributo allo studio dei nervi cutanei e delle terminazioni nervose nella oute e nella mucose orale dell' "Ammocoetes branchialis". Atti. R. Accad. Sci. Torino. 42.

— 1907 b. Sulla terminazione dei nervi nell'apparechio branchiale e nel velo boccale di Ammocoetes branchialis. Atti. R. Accad. Sci. Torino. 42.

FÜRBRINGER, M. 1897. Über die Spino-occipitalen Nerven der Selachier und Holocephalen. Festschr. C. Gegenbaur. 3. Leipzig.

GASKELL, J. F. 1912. The distribution and physiological action of the suprarenal medullary tissue in Petromyzon fluviatilis. J. Physiol. 44.

— 1914. The chromaffin system of annelids and the relation of this system to the contractile vascular system in the leech, Hirudo medicinalis. A contribution to the comparative physiology of the contractile vascular system and its regulators, the adrenalin secreting system and the sympathetic nervous system. Proc. Roy. Soc. Lond. 205.

— 1920. Adrenalin in annelids. A contribution to the comparative study of the origin of the sympathetic and the adrenalin-secreting systems and of the vascular muscles which they regulate. J. Gen. Physiol. 2.

GASKELL, W. H. 1920. The involuntary nervous system. New York.

GIACOMINI, E. 1902. Contributo alla conosenza delle capsule surrenali nei Ciclostomi. Monit. Zool. Ital. 13.

GOODRICH, E. S. 1927. The problem of the sympathetic nervous system from the morphological point of view. C. R. Ass. Anat. Paris. 22.

— 1930. Studies on the structure and development of vertebrates. London.

— 1937. On the spinal nerves of the myxinoidea. Quart. J. Micr. Sci. 80.

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HALLER VON HALLERSTEIN, V. 1934. Kranialnerven. In: Bolk, Göppert, Kallius, Lubosch: Handb. d. vergl. Anat. d. Wirbeltiere. Berlin, Wien.

HANSTRÖM, B. Hormones in invertebrates. Oxford.

HIRT, A. 1934. Sympathisches Nervensystem und Nebenniere. In: Bolk, Göppert, Kallius, Lubosch: Handb. d. vergl. Anat. d. Wirbeltiere. Berlin, Wien.

HÖRSTADIUS, S. 1950. The neural crest. London.

IHLE, J. E. W., VAN KAMPEN, P. N., NIERSTRASZ, H. F., VERRSLUYS, J. 1927. Vergl. Anat. d. Wirbeltiere. Berlin.

JOHNELS, A. G. 1955. Suppression of costaining of nonnervous tissue in protargol technics. Stain Techn. 30.

JOHNSTON, J. B. 1905. The cranial nerve components of Petromyzon. Morph. Jahrb. 34.

— 1908. Additional notes on the cranial nerves of Petromyzon. J. Comp. Neur. 18.

JORDAN, H. E. & SPEIDEL, C. C. 1930. Blood formation in cyclostomes. Amer. J. Anat. 46.

JULIN, C. 1887 a. Recherches sur l'appareil vasculaire et le système nerveux peripherique de l'Ammocoetes (Petromyzon planeri). Arch. Biol. 7.

— 1887 b. Le système nerveux grand sympathique de l'Ammocoetes (Petromyzon planeri). Anat. Anz. 2.

KAENSCHE, C. C. 1890. Beiträge zur Kenntnis der Metamorphose des Ammocoetes branchialis in Petromyzon. Zool. Beitr. A. Schneider. 2.

KEIBEL, F. 1927. Zur Entwicklungsgeschichte des Vorderdarmes und des Pancreas beim Bachneuauge und beim Flussneunauge. Zschr. Mikr.-Anat. Forsch. 8.

KOLMER, W. 1905. Zur Kenntnis des Rückenmarks vom Ammocoetes. Anat. H. 29.

KRAENZEL, F. 1933. Contribution à l'étude de la lamproie fluviatilis Lampetra (Petromyzon) fluviatilis. Arch. Biol. 44.

KRAUSE, R. 1923. Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. Berlin, Leipzig.

KUNTZ, A. 1945. The autonomic nervous system. Philadelphia.

LANDAU, E. 1956. Neuere Ergebnisse der Neurohistologie. Erg. der Medizinischen Grundlagenforschung. Stuttgart.

LANGERHANS, P. 1873. Untersuchungen über Petromyzon planeri. Verh. nat.-forsch. Ges. Freiburg. 6.

LICKTEIG, A. 1913. Beitrag zur Kenntnis der Geschlechtsorgane der Knochenfische. Zschr. Wiss. Zool. 106.

LINDSTRÖM, Th. 1949. On the cranial nerves of the cyclostomes with special reference to n. trigeminus. Acta Zool. 30.

MARCUS, H. 1910. Über den Sympathikus. Sitz.-ber. Ges. Morph. Physiol. München. 25.

MARINELLI, W. & STRENGER, A. 1954. Vergleichende Anatomie und Morphologie der Wirbeltiere. 1. Lampetra fluviatillis (L). Wien.

MÜLLER, J. 1839. Vergleichende Neurologie der Myxinoiden. Abh. Kön. Akad. Wiss. Berlin. 1838.

NICOL, J. A. C. 1952. Autonomic nervous systems in lower chordates. Biol. Rev. 27.

ONODI, A. D. 1886. Über die Entwicklung des sympathischen Nervensystems. II. Arch. Mikr. Anat. 26.

OWSJANNIKOW, Ph. 1865. Über die Inauguraldissertation des Herrn Kr. Kutschin das Rückenmark der Neunaugen betreffend, nebst einiger eigenen Beobachtungen über das Rückenmark der Knochenfische und anderer Thiere. Mélanges Biol. tirés du Bull. Acad. Imp. St. Pétersbourg. 4.

— 1883. Über das sympathische Nervensystem der Flussneunaugen, nebst einigen histologischen Notizen über andere Gewebe desselben Thieres. Bull. Acad. Sci. St. Pétersbourg. 28.

PALMGREN, A. 1948. A rapid method for selective silver staining of nerve fibres and nerve endings in mounted paraffin sections. Acta Zool. 29.

— 1955. Staining nerve fibres after sublimate-acetic and after Bouin's fluid. Stain Techn. 30.

PIETSCHMANN, V. 1933. Acrania—Cyclostoma Ichthya. In: Kükenthal, Krumbach: Handb. d. Zool. Berlin, Leipzig.

RANSON, S. W. & CLARK, S. 1947. The anatomy of the nervous system. Philadelphia.

RANSON, W. B. & THOMPSON, D'Arcy W. 1886. On the spinal and visceral nerves of Cyclostomata. Zool. Anz. 9.

RAZZAUTI, A. 1917. Struttura del tegumento dei Petromizonti. Nervi ed organi cutanei di senso. Arch. Ital. Anat. Embr. 15.

REISSNER, E. 1860. Beiträge zur Kenntnis vom Bau des Rückenmarkes von Petromyzon fluviatilis L. Arch. Anat. Physiol.

STIEDA, L. 1866. Referate aus der russischen Literatur. Arch. mikr. Anat. 2.

STUDNIČKA, F. K. 1895. Ein Beitrag zur vergleichenden Histologie und Histogenese des Rückenmarkes. (Über die sog. "Hinterzellen" des Rückenmarkes). Sitz.-ber. Ges. Wiss. Prag 37.

TRETJAKOFF, D. 1909. Das Nervensystem vom Ammocoetes. I. Rückenmark. Arch Mikr. Anat. 73.

— 1927. Das periphere Nervensystem des Flussneunauges. Zschr. Wiss. Zool. 129.

VOGT, C. und YUNG, E. 1889—1894. Lehrb. d. praktischen vergl. Anat. Brauschweig.

WHITING, H. P. 1948. Nervous structure of the spinal cord of the young larval brook-lamprey. Quart. J. Micr. Sci. 89.

YOUNG, I. Z. 1933. The autonomic nervous system of selachians. Quart. J. Micr. Sci. 75.

ZIESMER, Ch. 1951. Eine Verbesserung der Silber-Imprägnierung nach Bodian. Zschr. Wiss. Mikr. Tech. 60.

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EXPERIMENTS ON FORCED INTERRUPTION OF THE COPULATION IN MINK

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During the last one or two decades comprehensive statistical and experimental investigations concerning the physiology of reproduction in the mink have elucidated several peculiar phenomena, and the results have been of great advantage to the practical mink breeder. On the whole it can be said that the number of cubs in the litter has increased while the number of empty females has decreased as the result of more information about the breeding behaviour of the mink. However, there are still a lot of problems to be solved before our knowledge of the subject is satisfactory, even for practical purposes. In many ways the future work on the reproduction in the mink is of a more delicate nature and consequently requires more patient experimental work than hitherto. The value of results based on field trials and on data collected from mink farms lies in that they can be used to advise the breeder how to practise the matings, but also in picking out the problems which must be solved by careful experimental work.

It is well known that the mink differs considerably from the larger farm animals with respect to the reproductive process, e.g. the ovulation is dependent upon the mating, the mating season is extremely short, the length of the gestation period varies (relatively) 3 to 4 times more than for the horse, the cow and the sow, and so on. Concerning the copulation, the length of this act is tremendous. HANSSON (1947) observed the copulation for 227 matings and found a mean duration of 64 minutes, but the frequency distribution showed a pronounced skewness. About 50 per cent of the matings had a duration of less than one hour, while about 10 per cent endured for more than two hours. Further, it was observed that the duration of the copulation was correlated to the date of mating, the shortest time required was in the beginning of the season.

It seems also as if there is a difference between males, some males repeat a short mating while others make themselves conspicuous with a long lasting copulation. From a practical point of view it should be of great importance if the length of the matings could be shortened as it should be possible to utilize males with high breeding value more efficiently, and it should be possible to reduce the number of required males. It is known that some breeders have practised a forced interruption of the mating, but no detailed information of the influence of forced interruption on litter size, frequency of pregnancies etc. are available. The condition for that forced interruption of the copulation can be recommended is that the breeding result will be just as good as after spontaneous interruption. From a scientific point of view the effect of forced interruption must be divided into at least two different components before it can be analyzed: the influence on the number of spermatozoa ejaculated, and the influence on the number of eggs ovulated. Besides these two components others should be of great interest to analyze and e.g. to find out why the mink has adopted itself to a copulation act of such an extreme duration. However, this is out of the scope of this paper.

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In the present paper some results will be presented from experiments where the two mentioned components have been subjected to experimental analysis. The investigation was carried out during the years 1953—56. Valuable financial support was placed at our disposal by the Swedish Fur Breeders' Association to which we want to express our sincere thanks.

MATERIAL AND METHODS

Most of the females used in the experiments have been of the Standard type. During the first two years a few Silverblue females were also used, but no difference between the two types has been observed with regard to the reproductive processes. The males have been of different types too, most of them Standard type but a few Black Cross and Royal Pastel males have been used. No attention will be paid to the type in the treatment of the data.

All the males had good sexual drive and apparently good fertility (se later). The male fertility was determined by taking a vaginal sample from the female immediately after the mating was interrupted. If no sperms were found, or only dead sperms, the female was re-mated immediately after the first unsuccessful mating. Such cases, however, occurred only with females not included in the present experiments. Usually, the sample from the vagina was examined only for motile spermatozoa, first in the 1956 year's breeding season an attempt has been made to correlate the number of spermatozoa in the sample with the total number ejaculated.

When the females were killed the sex organs were removed and examined.

For collection of eggs and blastocysts the uteri were washed through with a physiological saline solution and the specimens collected in a watch glass. The ovaries were gently removed, care being taken not to squeeze possible corpora lutea. They were fixed in Bouins solution for histological examination afterwards. The sectioning of the ovaries was done on a thickness of 10 microns, the staining with Ehrlich's haematoxylin and erythrosin or eosin. Most of the sections were mounted and examined for corpora lutea, and in no cases more than 0.07 millimeter (of the ovary) was left out. By examination of most of the sections all the corpora lutea could be counted. The counting was made under microscope with free-hand drawings of every single corpus luteum in such a way that the individual body could be followed from the very first appearance until it disappeared.

RESULTS

1. THE RATE OF PASSAGE OF THE SPERMATOZOA THROUGH THE FEMALE REPRODUCTIVE TRACT

a. In a small experiment during the spring 1953 an attempt was made to determine the rate of semen transport from the *cervix* to the *bursa ovarica*. In earlier times it was a common belief that several hours elapsed before the spermatozoa were passed to the site of fertilization, but recent investigations on the cow (SALISBURY and VANDEMARK 1952) have unveiled that it is a matter of minutes only. In the mink ENDERS (1952) has reported that spermatozoa could be found at the tubo-uterine junction within 15 minutes after the beginning of the mating, but he was unable to find sperms beyond this point. Two hours after copulation spermatozoa were plentiful at the junction. The reason for that "sperm were not recovered from the uterine tubes nor the ovarian bursa" at 15 minutes has ENDERS (loc. cit.) "ascribed to faulty technique rather than to absence of sperm".

In the first experiment 6 males were used. For two males the copulation was interrupted 5 minutes, and for two males 15 minutes after the beginning of the mating, while the last two males were allowed to mate for half an hour. Immediately after the interruption of the mating a vaginal sample was taken from the female. In all cases living spermatozoa were recovered. One or two minutes later the females were killed and the sex organs removed. From one female in each of the three groups the uterus was cut in bits of about one centimeter length and washed through with physiological solution, while for the other female the uterus, cut up in the same way, was fixed for histological examination. The Fallopian tubes were fixed together with the ovaries.

The results from these observations are that spermatozoa could be found not only in the cervix 5 minutes after the onset of the mating, but also in the 1st

centimeter of the uterus. In the 2nd centimeter it was possible to find a few spermatozoa in one of the females in this group. No spermatozoa were found in the 3rd section. In the second group (interruption after 15 minutes' mating) it was shown that numerous spermatozoa were passed to the tubo-uterine junction. None were found in the Fallopian tubes. For the third group (30 minutes' mating) spermatozoa could be found throughout the tubes. For one of the females a few sperms were found in the bursa. The other female had only spermatozoa in the uterus and the tubes. In this connection it can be mentioned that ADAMS (1956) estimated the rate of spermatozoan transport in the rabbit to 0.5—3.3 mm per minute. Thus, the speed of the spermatozoa in the reproductive tract of the mink is about the same as in the rabbit, and far less than in the cow. Whether the action of the uterus is of secondary importance as assumed for the rabbit or the uterus takes direct part in the transport as for the cow and other species is not known.

b. In 1956 a new experiment was carried out with the scope of studying the number of spermatozoa ejaculated in connection with the length of the mating act. For this purpose 12 females of Standard type were grouped randomly in three groups for mating 6, 12 and 24 minutes respectively. The females were subjected to laparotomy in the beginning of the mating season and a ligature was placed at the tubo-uterine junction. In the last third of the season the females were mated only once according to the schedule. Within every group two females were killed immediately after the forced interruption of the mating, while the two others were killed half an hour after the beginning of the mating. This means that the time available for the last ejaculated spermatozoa for passage through the female reproductive tract was 24, 18 and 6 minutes for the three groups respectively. Immediately after the animals were killed, the sex organs were dissected out and the uterine horns were cut in parts of approximately one centimeter of length (as in the 1953 year's experiment). No sections were fixed, but all were stored in 0.5 cc physiological saline solution.

For estimation of the number of spermatozoa the different sections were washed through with 1 cc saline solution (0.5 cc storage fluid + 0.5 fresh saline solution) several times in both directions. The counting of the numbers was made in a Thomas—Bürker chamber. At least 2 times 144 squares were counted from every sample. The results from this experiment are presented in tables 1 and 2.

From Table 1 it is seen that there is a tremendous variation within the three groups, but on the whole there is an increasing number of spermatozoa with increasing duration of the copulation. However, as will be demonstrated in a later chapter, the number of spermatozoa which a male ejaculates during 12 minutes' mating is generally sufficient for normal fertilization of the eggs, while a mating of only six minutes' duration will in most cases not be long enough, i.e. the number of fertilized eggs will be less than for a longer lasting mating

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TABLE 1. *Number of spermatozoa after different duration of the copulation.*

Group	Female no.	Male no.	Date of mating	Total number of sperms (in thousands) in uterus	Points for sperms in the vaginal sample
I : 1	3	E 22	19/3	0	2
	8	C 172	28/3	231	2
I : 2	9	H 528	19/3	48	1
	14	D 69	28/3	4	2
Mean				71	1.75
II : 1	12	C 172	21/3	10 627	3
	10	C 174	28/3	7 916	5
II : 2	5	C 174	16/3	57	1
	7	C 174	23/3	7	4
Mean				4 652	3.25
III : 1	11	H 507	21/3	15	4
	6	C 172	27/3	1 536	3
III : 2	4	C 172	19/3	6 472	5
	13	C 174	26/3	17 814	4
Mean				6 459	4.0

Subgroup (: 1): The females were killed immediately after the interruption of the mating.
Subgroup (: 2): The females were killed half an hour after the beginning of the mating.

TABLE 2. *Mean number of spermatozoa per uterine section and in cervix (in thousands).*

Group	Section	Subgroup 1* Left side	Subgroup 1* Right side	Subgroup 2 Left side	Subgroup 2 Right side
I	4	5.2	13.0	—**	—
	3	3.1	11.3	0.5	0.9
	2	3.1	20.9	1.3	3.1
	1	7.0	17.4	0.9	4.8
	Cervix	43.9		14.8	
II	4	—	—	—	2.6
	3	1 142.1	1 957.9	0.5	1.3
	2	833.4	1 597.3	2.6	5.2
	1	1 402.4	1 119.4	4.9	5.2
	Cervix	1 219.2		9.8	
III	4	—	—	—	—
	3	116.3	106.3	658.0	1 875.9
	2	144.2	89.0	1 879.3	2 532.2
	1	87.3	105.0	1 434.7	2 437.0
	Cervix	128.5		1 655.0	

* 2 females in each subgroup, cf. Table 1.
** A dash means that the uterine horns were divided in 3 sections only due to a smaller length.

act. In some cases a male will deposit a large number of spermatozoa immediately after the beginning of the mating, and cases are not uncommon in practical mink breeding where a copulation of less than 6 minutes has resulted in a litter of approximately normal size.

The male C 172 e.g. in the first group (6 min.) has probably ejaculated enough spermatozoa for achieving maximum fertilization rate. This particular male was used only the four times given in the table. He showed very good fertility in all the matings. The male no. C 174 showed a high fertility in the last two matings. (In addition, he was used two times not recorded in the table.) No. H 528 was used only for the single mating on the 19th of March during the whole mating season. Male no. H 507 had mated on two consecutive days before he was used in the present experiment, while no. D 69 had had a rest of 5 days before the experimental mating. The male E 22 was more used. He had mated every day from the 8th until the 19th with exception of the 10th and the 14th before the experimental mating. Further, he had mated a female for 70 minutes on the very same day he was used in the experiment. He was used again because he was the only male who showed sexual interest in that particular female. However, the number of spermatozoa found in the vaginal sample was rather high, and indicated that a normal mating had taken place. That no spermatozoa could be found in the cervix or the uterine horns suggests an abnormal sexual behaviour of the female. Perhaps she had passed the top of the oestrus despite she allowed mating. As mentioned, no other males would mate her.

Concerning the two different time intervals when the females were killed after mating, nothing has been found which indicates a different distribution in the different sections, i.e. there is no indication of an accumulation of spermatozoa at the tubo-uterine junction with half an hour interval between the beginning of the copulation and the death of the female. The reason for this can be that the main part of the spermatozoa need longer time for the passage through the uterus, and it is only the "vanguard" which can be found at the junction (or in the tube or bursa, cf. 1953 years's experiment) after half an hour.

The results from the sperm countings in the different sections of the uterine horns and the cervix are presented in Table 2. An interesting matter is that generally a higher number is found in the right horn than in the left horn. The biological significance of this is difficult to understand. Perhaps there is a connection between the distribution of the spermatozoa and the length of the horns, the right being longest.

As mentioned previously semen control is performed on all females. The sample is taken with a thin glass pipette. A few drops of a physiological saline solution is injected into the vagina before the sample is sucked into the pipette. At the microscopic examination a point scale is used for classifying or esti-

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mating the number of spermatozoa. It was found that one plus corresponded to less than 100,000 spermatozoa, two plus from 100.000 to 300,000, three plus from 300,000 to 700,000 and four plus to about 800,000 spermatozoa per cubic millimeter of the sample. (The maximum of 5 plus has not yet been estimated.) The values given here are only preliminary ones. More work on this line will be laid down in the next year's experiments, before a scale for practical use can be worked out. However, by glancing over Table 1, it will be seen that in general there has been a fairly good agreement between the values of the vaginal sample and the numbers found in the uterus. The correlation has been found to $r = 0{,}51$ ($0.05 < P < 0{,}1$, the number is only 12). On an average the first group has got 1.75 plus, and shows a mean of 71,000 spermatozoa, the second group has got 3.25 plus with a number of 4,652,000 spermatozoa, while the third group got 4.0 plus on an average with 6,469,000 spermatozoa. These figures represent the total number found in the cervix and throughout the uterine horns. To be able to compare the concentration of spermatozoa in the uterus with the concentration in the vaginal sample, the total number must be reduced to the concentration per cubic millimeter. By doing this it is found that the first group has a concentration of 88 sperms, the second group shows 620 sperms and the third group 9569 spermatozoa per cubic millimeter. The much lower concentration in the uterus compared with that in the vagina demonstrates "The ability of the cervix to restrict the passage of the sperms into the uterus" in much the same way as found for the rabbit (BRADEN 1953). In our material it is not possible to compute the proportion of sperms which pass the cervix as the total number ejaculated is unknown, only a very rough estimate can be made. If it is assumed that the sample from the vagina really is a measure of the spermatozoan content of the vagina, an estimate of the total ejaculated number of sperms can be computed by multiplying the concentration with 94. The amount of saline which is injected into the vagina is namely 94 ± 1 cubic millimeter. Further, it is assumed that the points of 1.75 (group I) indicates a concentration of 175,000 sperms, 3.25 points (group II) correspond to 725,000 sperms, and 4.00 points (group III) to a concentration of 800,000 sperms. The total ejaculated number of spermatozoa will then be the sum of the above mentioned product and the number found in the cervix and the uterine horns. For the three groups the following figures are arrived at:

	Total number	Number in uterus	Ratio
Group I	16.5 millions	0.05 millions	330:1
„ II	73 „	4.0 „	18:1
„ III	82 „	6.5 „	13:1

The specious matter with the figures is that they fit well with the results found by CHANG (1951) who showed that only when rabbits were inseminated with an abnormally low number of sperms the number which reached the Fallopian

tubes was reduced. The same is the case for group I, with respect to the number which has passed the cervix. BRADEN (1953) found that only one sperm of every 40 sperms ejaculated would pass the cervix in the rabbit, while the figures computed for the present material points to one of every 10—20 with a reasonable duration of the copulation. As stressed by BRADEN differences exist between species. It is therefore probable that the number of sperms which pass the cervix is relatively high for a species as the mink, where the copulation is a long lasting process. Another point which must be mentioned is, that BRADEN (1953) found an increase in the number of spermatozoa in the uterus and the tubes with an increase in the interval between the mating and the slaughter until 4 hours after the mating. In our material the animals were killed half an hour after the beginning of the copulation, so therefore no accumulation of spermatozoa at the tubo-uterine junction has been found. How many of the spermatozoa which will reach the site of fertilization can not be estimated in the present material, as no spermatozoa could enter the tubes. It is admitted that a reiteration of the experiment—with some alterations in the technique—is desirable.

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2. INTERRELATION BETWEEN DURATION OF COPULATION AND FERTILITY

With the scope of studying the influence of the duration of the mating act on the fertility a series of experiment was started in the spring 1954 and continued in 1955. Here, it was not only the male fertility which was examined by measuring the number of fertilized eggs, i.e. if sufficient number of spermatozoa was ejaculated for achieving maximum fertilization, but also the stimulating power of the copulation on the rate of ovulation was subject to analyses. The experiment was designed so that one group of females should be mated for only 6 minutes, a second group for 12 minutes while a third group should be mated for 24 minutes, i.e. according to the same schedule as was adopted in the 1956 year's experiment with sperm examination. However, as there was a difference in the technical procedure (se later on for details) between the experiment in 1954 and the experiment in 1955, the results will be presented separately for the two years. In both series, the measurement of fertility was the number of fertilized eggs and living blastocysts. These were collected from the uterus by washing it through with a physiological saline solution at a certain number of days after the mating.

a. In 1954, 15 females, 12 Standard and 3 Silverblue, were used in the experiment, only one female did not mate. In the group I four of the females mated 6 minutes, whereafter the mating was interrupted by force, 1 female mated in 4 minutes only (spontaneous interruption). In the third group only two females were mated according to the plan (24 minutes), the three others had spontaneous interruption after 21, 23, and 24 minutes respectively. With the

TABLE 3. *Number of eggs, blastocysts, and corpora lutea from females in 1954 year's experiment.*

Group	Female no.	Date of mating	Number* of eggs	Number of blasto-cysts	Number of corpora lutea	Notes
I	4	15/3	0+0	0+0	4+7	
	(11	17/3	0+0	0+0	0+0)	Infection in left ovary
	13	19/3	0+0	5+4	5+4	
	(9	24/3	0+0	(?)	12+6)	4 min. mating, implantation
	14	26/3	0+0	0+0	8+4	
Total			0	9	32	
II	6	15/3	0+0	5+4	8+9	
	12	16/3	0+0	0+0	1+6	
	2	17/3	0+0	7+3	7+4	
	8	18/3	0+5	2+0	8+5	
Total			5	21	48	
III	5	10/3	0+0	0+0	6+5	23 min. mating, killed 11 days after mating
	1	15/3	0+3	2+5	2+8	
	7	16/3	0+0	1+6	5+12	24 min. mating, spontaneous interruption.
	10	18/3	0+0	6+5	9+5	21 min. mating, spontaneous interruption.
	(3	24/3	0+0	(?)	11+2)	implantation
Total			3	25	52	

* In the columns are the data presented individually for the left and right ovary and uterine horn.

exception of two females, mated on the 10th and the 15th of March, which were examined 11 days after the mating, all the females were killed 21 days after the copulation. The reason for the change to a collection of blastocysts at a later stage was that it was decided to use the blastocysts for chromosome studies. The first successful collection (cf. Table 3) gave blastocysts which were too small, and it was believed that 3 week old specimens would be more suitable. However, it soon appeared that also at this stage most of the blastocysts were resting, no mitotic divisions (or very few) were detectable, and therefore the material was unsuitable for cytological work. To avoid too many changes in the plan, the interval between mating and collection of blastocysts was not changed back again. This was unfortunate, because two of the females, mated late in the

season had early implanted foetuses (the exact number could not be determined), so in these cases the collection was too late.

The results with regard to the number of eggs, blastocysts and corpora lutea from this series are presented in Table 3.

By examination of the table it is immediately revealed that a total of only 8 eggs was found. This was undoubtly due to the late date of collection of eggs, or more correct, the large interval between mating and collection (cf. next series). However, all the eggs were dead, in one case 11 days (female no. 1), in the other case 21 days after mating (female no. 8). In both cases the eggs were found together with living blastocysts. The total number of blastocysts was 55. In one female the left ovary was infected and therefore no ovulation could be expected. In two other females implantation of the blastocysts had taken place and—as mentioned—no exact count of implantation sites could be done. If these three females are discarded the average number of blastocysts will be 5.0. However, the 55 blastocysts were found from only 7 females, that is the average "litter size" is 7.86 for "pregnant" females.

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The number of corpora lutea is also presented in the table. If it assumed that the number of corpora lutea is a criterion of the number of eggs released from the ovary, the ovulation rate is 12.0 eggs per female (no:s 11, 9 and 3 are omitted), and the average survival is 41.7 per cent. The mean number of corpora lutea for 13 females (only no. 11 omitted) is 12.54. For the seven females, from which living blastocysts were found, the number of corpora lutea is 91 in all, which means that 60.4 per cent of the eggs have developed to blastocysts. The uncertainty in these computations is that it does not seem legitimate to put a sign of equation between the number of eggs and the number of corpora lutea, at least for the mink. ENDERS (1952) found that about 10 per cent of the follicles which developed to the ripe stage did not rupture. Some of them could be converted into a corpus luteum like body. This observation is confirmed by our results through the findings of a corpus luteum in which remnants of the eggs could be seen.

Another reason for the high number of corpora lutea could be that a spontaneous (nervous) ovulation had occurred early in the mating season, before the experimental mating was done.

By comparing the results from the three groups it is seen that no difference exists between the groups which were mated for 12 and 24 minutes. The counted number of blastocysts was 21 in group II and 25 in group III (four females in each group of which 3 in each had blastocysts), while in group I only one female had living blastocysts.

Based on the demonstration of corpora lutea it is found that all the normal females have responded to the copulation.—It is admitted that the number of animals in the individual groups is very small.

b. In the experiment carried out in 1955, 30 females were placed at our dis-

posal. They were all second-year Standard females. As it is very important for the interpretation of the results that the females were mated only once, and that no ovulation was induced by unsuccessful mating (riding) or by other false stimuli, a few females had to be discarded. Further, two females did never show any signs of oestrus, and have therefore also been left out. A *post mortem* examination in the beginning of April revealed severe underdevelopment of the uterus in one case, while in the other case the appearance of the uterus pointed towards approaching oestrus. No morphological abnormalities could be detected in this case. A total of 25 females was mated according to the experimental design. The results are given in Table 4.

In the first group 9 females were set in, three of which had spontaneous interruption of the copulation after 2, 3 and 6 minutes respectively. Group II and group III comprised 8 females each. Female no. 13 should have mated for 24 minutes, but was included in group II. No. 21 in this group had spontaneous interruption after 12 minutes. In the third group only two females had spontaneous interruption (21 and 20 minutes). The animals were killed 11 days after mating with the exception of two animals which were killed 12 days after mating.

It is seen from the table that the most important difference between the groups is that in group I living blastocysts were found from only one female, contrary to the two other groups where 6 and 5 females respectively were "pregnant". In the first group one female had pathological modifications in the left ovary (cf. 1954-years series), but else all the other females in this group had ovulated if the existence of corpora lutea is taken as an indicator. In group III female no. 27 had only one corpus luteum, but in this particular case it was clear that no ovulation had occurred, because remnants from the ovum was visible in the center of the corpus. If the two mentioned females are omitted, the mean number of corpora lutea is 12.09 ± 0.88, which is not significant different from the mean found the year before (12.54 ± 0.91), nor is there any difference between the three groups. It can also be mentioned that no difference has been detected between the right and the left ovary in the number of corpora lutea, and no difference has been found in the number of eggs plus blastocysts washed out from the left and right horn of the uterus.

While blastocysts were found in only one female in the first group, eggs were recovered from five other females. However, all these eggs had died at the time of examination. This means that only one out of 8 females could be regarded as pregnant. In the two other groups, one female in each had not ovulated, when the recovery of eggs or blastocysts is taken as the criterion. Usually dead eggs were found together with the blastocysts, but for three females, no:s 21, 11 and 22, only dead eggs were found. The reason for this is—with high probability—that the two males used to these three females were sterile. The appearance of the eggs pointed towards unfertilized eggs, and a

TABLE 4. *Number of eggs, blastocysts, and corpora lutea from females in 1955 year's experiment.*

Group	Female no.	Date of mating	Number* of eggs	Number of blasto-cysts	Number of corpora lutea	Notes
I	(2	11/3	0 + 0	0 + 0	0 + 0)	Inflammation in left ovary.
	5	11/3	0 + 0	4 + 5	6 + 6	
	14	15/3	4 + 0	0 + 0	11 + 6	Spontaneous interruption, 6 min.
	15	21/3	4 + 3	0 + 0	7 + 8	3 min. mating
	26	21/3	7 + 3	0 + 0	8 + 5	2 min. mating
	23	22/3	3 + 3	0 + 0	3 + 6	
	16	24/3	0 + 0	0 + 0	2 + 2	
	1	26/3	0 + 0	0 + 0	6 + 7	
	29	26/3	4 + 2	0 + 0	7 + 8	
Total			33	9	98	
II	10	8/3	3 + 1	5 + 3	10 + 6	
	18	8/3	0 + 0	0 + 0	3 + 2	
	28	15/3	0 + 1	3 + 0	5 + 2	
	13	16/3	0 + 0	2 + 3	7 + 4	14 min. mating, spontaneous interruption
	21	16/3	7 + 5	0 + 0	7 + 6	
	24	22/3	0 + 2	0 + 2	9 + 6	
	30	22/3	1 + 3	3 + 3	8 + 9	
	25	24/3	0 + 0	2 + 6	4 + 8	
Total			23	32	96	
III	7	15/3	2 + 0	2 + 4	5 + 5	20 min. mating, spontaneous interruption
	11	15/3	4 + 7	0 + 0	6 + 7	
	27	15/3	0 + 0	0 + 0	0 + 1	21 min. mating, spontaneous interruption
	9	18/3	0 + 0	3 + 4	4 + 6	
	22	18/3	1 + 9	0 + 0	1 + 12	
	17	19/3	0 + 0	2 + 3	2 + 4	
	4	22/3	0 + 3	2 + 3	10 + 12	
	3	26/3	3 + 1	1 + 0	5 + 5	
Total			30	24	85	

* In the columns are the data presented individually for the left and right ovary and uterine horn.

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controll of other females (not in experiments) which had been mated to the same two males, revealed that none of them got pregnant. This was a discouraging finding, as the sperm test was positive for both males. For comparison of the pregnancy result between the different groups it seems therefore valid to leave these females out. The number of "pregnancies" will then be 1 out of 8 in group I, and 11 out of 13 for groups II and III pooled. For practical use, the computations must be based on all the mated females. The results will then be that after a mating of short duration only one out of 9 females got "pregnant", while 11 out of 16 females could be regarded as pregnant after matings of 12 minutes (or longer) duration, when only one mating is practised.

The reason for the very low pregnancy result in the first group seems to be that the males have not ejaculated sufficient numbers of spermatozoa, but it is difficult to distinguish between too few spermatozoa and a failling semen transport. With reference to the experiment in 1956 concerning the number of spermatozoa ejaculated, it seems legitimate to conclude that in general it is the low number of spermatozoa which is the cause of sterility or barrenness following a copulation of short duration. This point of view is supported by the fact that eggs or blastocysts were found from 6 out of 8 normal females. If one relies upon the finding of corpora lutea all the females had ovulated. Of a total of 42 eggs, however, only 9 eggs developed to blastocysts, and they were all from the same female, cf. the large number of spermatozoa ejaculated by male no. C 172. That the ovulation rate is influenced too, will be discussed later on.

With regard to the other groups (II and III) there are ni differences concerning the number of eggs and the number of blastocysts, which can be ascribed to the difference in the duration of the copulation. In the second group 41.8 per cent of the ovulated eggs did not continue the development to the blastocyst stage, for group III the figure is 55.5 per cent, when the computations are made on the total number of eggs and blastocysts. But if the unfertilized eggs are excluded, the figures are 25.6 and 27.3 per cent, respectively. The mean number of ovulations (eggs plus blastocysts) for the pooled data is 6.81. Only 3.50 eggs on an average developed to blastocysts, that is a gross embryonic mortality on an early stage of 48.6 per cent. For the 11 females, where the matings can be regarded as normal, the mean number of ovulations is 6.91, and the mean number of blastocysts is 5.09. This means that under normal conditions with only one mating the early embryonic mortality amounts to 26.3 per cent.

An estimate of the number of follicles that did not rupture can be arrived at by comparing the number of eggs and blastocysts with the number of corpora lutea. The only female, which it is legitimate to leave out in this case, is no. 2. For the remaining 24 females the mean number of corpora lutea is 11.63 ± 0.96 with a corresponding number of eggs and blastocysts of 6.29 ± 0.77. This

should mean that 45.9 per cent of the follicles had not ruptured, although they developed into corpora lutea. There is no doubt that this figure is far too high, and that more investigations are necessary to find out to what extent unruptured follicles are transformed to corpora lutea and to what extent a non-copulatory ovulation is involved. On the other hand, if it is assumed that other factors than the mating, influence the three groups equally, some information can be gained with regard to the influence of the duration of the mating on the proportion of unruptured follicles. The results are set up in Table 5.

TABLE 5. *Average number of "unruptured" follicles, including corpora lutea from non-copulatory ovulations.*

Group	Number of unruptured follicles	Number of corpora lutea	Percentage of unruptured follicles
I	7.00	12.25	57.1
II	5.12	12.00	42.7
III	3.88	10.63	36.5
II + III	4.50	11.31	39.8

With respect to the number of corpora lutea, no differences can be found between the three groups. Concerning the number of "unruptured" follicles there is no difference between group II and group III, so it seems valid to pool the groups. On an average, a prolongation of the copulation from 6 to at least 12 minutes will cause 2.5 follicles more to burst. When comparison is made between the three groups with regard to "unruptured" and ruptured follicles a χ^2 test shows that a significant difference exists between group I on the one hand and group II and III on the other ($\chi^2 = 8.43$, $0.01 < P < 0.02$). It seems therefore legitimate to draw the conclusion that the poor breeding result achieved with a short duration of the copulation is not only due to an insufficient number of spermatozoa (in general), which is stressed by the significant difference in the proportions of dead eggs (unfertilized) to blastocysts between the groups ($\chi^2 = 13.00$, $0.001 < P < 0.01$), but also to a subnormal stimulation of the reproductive process necessary for the maximum activation of the ovary.

DISCUSSION

One of the main problems in the practical mink breeding is the high frequence of barren females, which is very often found. Figures of 25—30 per cent are not uncommon. The causes for barrenness can be many, but the most

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important ones can probably be divided into two classes, the first of which comprises disturbances in the hormonal system, including failure of ovulation, and the occurrance of a periodic or permanent sterility in some males. The other class will then include the heavy loss of foetuses during the gestation period. Concerning the embryonic mortality, or more correct the pre-natal loss, only limited data are available for the mink with regard to the quantity, and nothing —so to say—is known about the causes of the loss. Further, it is not known to what extent the death affects the whole litter more frequent than single foetuses. HANSSON (1947) found for 21 females that 7.29 foetuses had implanted during the first part of the gestation period, and from a material comprising 2380 births he computed the mean litter size to 4.37 kits. When based on the number of corpora lutea (8.73 per female) he found that 1.44 eggs or blastocysts died before implantation, and arrived at the conclusion that a total of 50 per cent of the released ova succumb during the gestation. From the material of ENDERS (1952) the pre-natal death can be estimated to about 55 per cent. In the present material the data from group II and III in 1955 point to a loss before implantation of nearly 30 per cent (11 females with eggs and blastocysts or blastocysts only), a loss which is about twice that found by HANSSON (1947). The total loss of eggs, including unfertilized eggs from three females, amounts to 49 per cent, and if the first group is included the loss rises to 57 per cent. For the first group it seems probable that the majority of the eggs have been unfertilized. The only way to avoid barrenness for females mated for only a few minutes is to remate latest the day after the short mating.

It was mentioned that the total loss from ovulation until the blastocysts stage was found to be 49 per cent for group II and group III in 1955 year's experiment. When the material for the two years is pooled, and the computation is done on the number of corpora lutea for females in which blastocysts were found (all the groups) the loss is estimated to 50 per cent. There is, however, one matter, which must be emphasized, viz. that the number of corpora lutea found in the present material is considerably higher (11.95 ± 0.70, $n = 37$) than that found by ENDERS (1952). He found a higher number (9.8) than HANSSON (1947) (8.73 ± 0.30). The reason for the higher findings in the more recent investigations is unknown. That the fertility has increased during the last decade is clear. VENGE (1953) found a mean litter size for Standard mink of 5.02 kits and 5.08 kits for mink of the Pastel type. JOHANSSON (1955) found 5.25 and 5.06 kits per litter for the same two types. But the increase from 4.37 kits found by HANSSON (1947) to the mentioned mean litter size found some 10 years later, cannot explain the difference in the number of corpora lutea. At least two different explanations can be offered, the one is that the mink kept in captivity is undergoing a change in the reproductive processes, so that e.g. spontaneous ovulation is more frequent now than 4—6

generations ago, but this seems improbable. One should on the contrary regard it for more probable that the longer time which elapses under captivity, the more tame will the mink be, and therefore less influenced by environmental disturbances (handling, sudden noise etc.). The other explanation is that there has been a difference in the way in which the countings have been made. In our work not more than 0.07 millimeter of an ovary has been left out in any case.

Despite the fact that the number of animals involved in the present experiment is rather low, it seems justified to draw the conclusion that a copulation of only 6 minutes duration is too short for achievement of a satisfactory breeding result. In a few cases pregnancy will occur, but generally should females, mated in that way, be remated immediately, or latest on the day after. It is believed that the short duration of the copulation will not expose the female to the maximum stimulation of the ovulation mechanism with the result that the rate of ovulation will be less (2—3 eggs) than for females mated for 12 minutes or longer. However, it was also demonstrated that most of the females—at least all the normals—had ovulated after a 6 minutes' mating, and this fact stresses the ease with which the ovulation can be aroused. The vast majority of the eggs, however, were unfertilized, apparently because too few spermatozoa were ejaculated or because of failure in the passage of the sperms. Compared with the findings from the sperm experiment in 1956 the first explanation looks most plausible. The sperm experiment also gives the explanation why pregnancy occurs in a few instances. Females which have had a short mating act should therefore be remated the day after so that a new dose of semen can be transported to the site of fertilization before the eggs are released from the ovary, but also to induce a further activation of the gonadotrophic principles involved in the growth and maturation of the follicles. HANSSON (1947) claimed that the ovulation followed 36—37 hours after mating, while ENDERS (1952) reported that ovulation took place 48 hours after mating. Further, he stated that the follicles did not rupture at one time. He found that ovulation took place between 42 and 52 hours after the copulation. This observation provides an explanation for the increase in litter size if a female is remated with one day's interval, and supports the hypothesis that a further stimulation (mating) is favourable for the ovary so that more eggs will be released. In fact, it is in agreement with the results from the present investigation, although it is not known how much the increase in litter size would be if a female mated for only six minutes the first day, was remated the day after. From JOHANSSON (1953) the following figures can be quoted as an example of the increase in litter size due to remating. In addition figures are also quoted, showing the decrease in empty females, which matter is more depending upon the date of mating than on remating.

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TABLE 6. *The influence of remating upon the litter size and the percentage of empty females* (from JOHANSSON 1953).

Date of beginning of mating	One mating	Remating, interval: 1 day	7—10 days
10th of March:			
Litter size	4.31	4.75	5.38
Empty females, pCt.	42	36	17.5
20th of March:			
Litter size	4.65	5.03	
Empty females, pCt.	20	13	

The figures for empty females support very strongly the hypothesis that the main cause of a heavy pre-natal loss is the delayed implantation. The most important thing for the practical breeder is to get as many matings as possible in the final third of March. The figures indicate that with regard to the loss before implantation the destruction of the whole litter is the serious factor in the pre-natal mortality. The increase in litter size with mating only once from the early to the late date of mating points to a death within the litter of one third kit on an average. The increase from mating once to remating the day after is the same (about 0.4 kit on an average) for the two dates of mating. The proportion of empty females for matings in the final third of the season, thus including females mated for the first time on the 10th, is only half of that in the beginning, either these have been mated only once or remated the day after. The increase in litter size for females remated 7—10 days after the first mating is to be ascribed to fact that 14—15 per cent of the ova from the first mating will survive a remating (JOHANSSON and VENGE 1951) with an interval of more than 6 days.

SUMMARY

During the years 1953—1956 some experiments were carried out with mink with the aim of studying the relation between the duration of the copulation and the fertility. Two types of experiments have been run, one concerning the semen passage through the female reproductive tract including the number of spermatozoa ejaculated, the other concerning the rate of ovulation. For three groups the matings were interrupted by force after 6, 12 and 24 minutes. By co-ordinating the results the following conclusion is drawn. A mating of only 6 minutes' duration is too short to obtain normal breeding result, due not only

to insufficient number of spermatozoa ejaculated, but also to less stimulation of the female, so that the number of eggs released from the ovary is less, than after a mating of 12 minutes or longer duration. By forced interruption of the copulation when 12 minutes have elapsed since the intromission, it is possible to utilize the males more intensively, thus reducing the number of males kept over. No difference between copulation for 12 or 24 minutes could be demonstrated.

LITERATURE CITED

Adams, C. E. (1956). A study of fertilization in the rabbit: The effect of post-coital ligation of the Fallopian tube or uterine horn. — Jour. Endocrinol., *13*, 296—308.

Braden, A. W. H. (1953). Distribution of sperms in the genital tract of the female rabbit after coitus. — Austral. Jour. Biol. Sci., *6*, no. 4, 693—705.

Chang, M. C. (1951). Fertilization in relation to the number of spermatozoa in the Fallopian tubes of rabbits. — Ann. Ostet. e Ginec., *2* fasc. spec., no. 7, 918—925.

Enders, R. (1952). Reproduction in the mink (Mustela vison). — Proc. Amer. Phil. Soc., *96*, no. 6, 691—755.

Hansson, A. (1947). The physiology of reproduction in mink (Mustela vison, Schreb.) with special reference to delayed implantation. — Acta Zool., *28*, 1—136.

Johansson, I. (1953). Parningsmetodens inflytande på standardminkens avelsresultat. — Våra pälsdjur, *24*, no. 3, 48—54.

— (1955). Mutationsminkens avelsresultat och storlek i jämförelse med standardmink. — Våra pälsdjur, *27*, no. 2. 23—31.

— and Venge, O. (1951). Relation of the mating interval to the occurrence of superfetation in the mink. — Acta Zool., *32*, 255—258.

Salisbury, G. W. and VanDemark, L. L. (1952). Physiological basis for site of semen deposition and procedure in artificial insemination of cattle. — Rep. IInd Int. Congr. Physiol. & Pathol. Anim. Repr. & Artificial Insem., *III*, 99—106.

Venge, O. (1953). The sex ratio in farm mink. — Acta Zool., *34*, 294—302.

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